How to analyze exercising ecgs

Schwarzwald, Colin C

HOW TO ANALYZE EXERCISING ECGS

Colin C. Schwarzwald
Prof. Dr. med. vet., PhD, Dipl. ACVIM & ECEIM
Zurich, Switzerland

Introduction

Exercise testing is an important component of cardiovascular assessment and includes continuous recording of an ECG using a device that has permanent storage and playback capabilities. Recording quality is crucial for subsequent analyses of the ECG tracing, since excessive motion artefacts or lead failure can severely impair the diagnostic quality of a recording. The methods used for recording an exercising ECG will be covered in another presentation at this meeting. This session will focus on analyzing the ECG tracing once the exercise test is completed.

While analyzing a stress ECG, the clinician has to keep in mind the main questions that need to be answered. These are usually focused on defining the safety risks to the horse and to the rider or driver, which is paramount in horses with cardiovascular disease. Safety risks may be linked to inappropriate exercising heart rates, aberrant conduction, or ectopy associated with physical activity and adrenergic stimulation. Of principal concern are the hemodynamic consequences of rate and rhythm disturbances (hypotension, low cardiac output, poor peripheral perfusion) and the potential for further electrical destabilization (malignant, potentially fatal arrhythmias). Generally, ventricular arrhythmias have a greater potential impact on safety compared to supraventricular arrhythmias.

Stepwise Approach to Analyze Exercising ECGs

Analyzing exercising ECGs can be challenging, and a stepwise approach is recommended. In the following, analysis of exercising ECGs will be described using the following software:

- For ECG analysis and export of RR interval series:
    - Televet (Engel Engineering Service GmbH, Offenbach, Germany, www.televet.de)
- For graphical presentation of RR and HR time series and for advanced statistical calculations:
    - Microsoft Excel (Microsoft Corporation, Redmond, USA)
    - Kubios HRV (Biosignal Analysis and Medical Imaging Group, University of Eastern Finland, kubios.uef.fi)
    - Graph Pad PRISM (GraphPad Software, La Jolla California USA, www.graphpad.com)

While these software packages are widely used by many clinicians, the basic principles of exercising ECG analyses can also be applied using other type of equipment and analysis software. Even if older, analogue equipment without digital analysis options is used, the same general principles apply.

1. Check quality of recording

Artefacts caused by electrical disturbance, motion, twitching, muscle tremor, improper lead placement or equipment failure can severely impair the diagnostic value of an ECG recording. Therefore, as a first step, the quality of a stress ECG recording should be assessed. In fact, if possible (i.e., if telemetric ECG equipment is used), quality assessment should be done during exercise testing and throughout the recovery period while the equipment is recording. This allows fixing of the lead positioning and detecting potential equipment failure and ensures high quality recordings. If the ECG recording is of insufficient quality, one should consider repeating the exercise test to allow a proper

diagnostic assessment. Once the ECG tracing is loaded into the analysis software (i.e., Televet), quality control can be performed using the "overview" screen (**Figure 1a/b**).

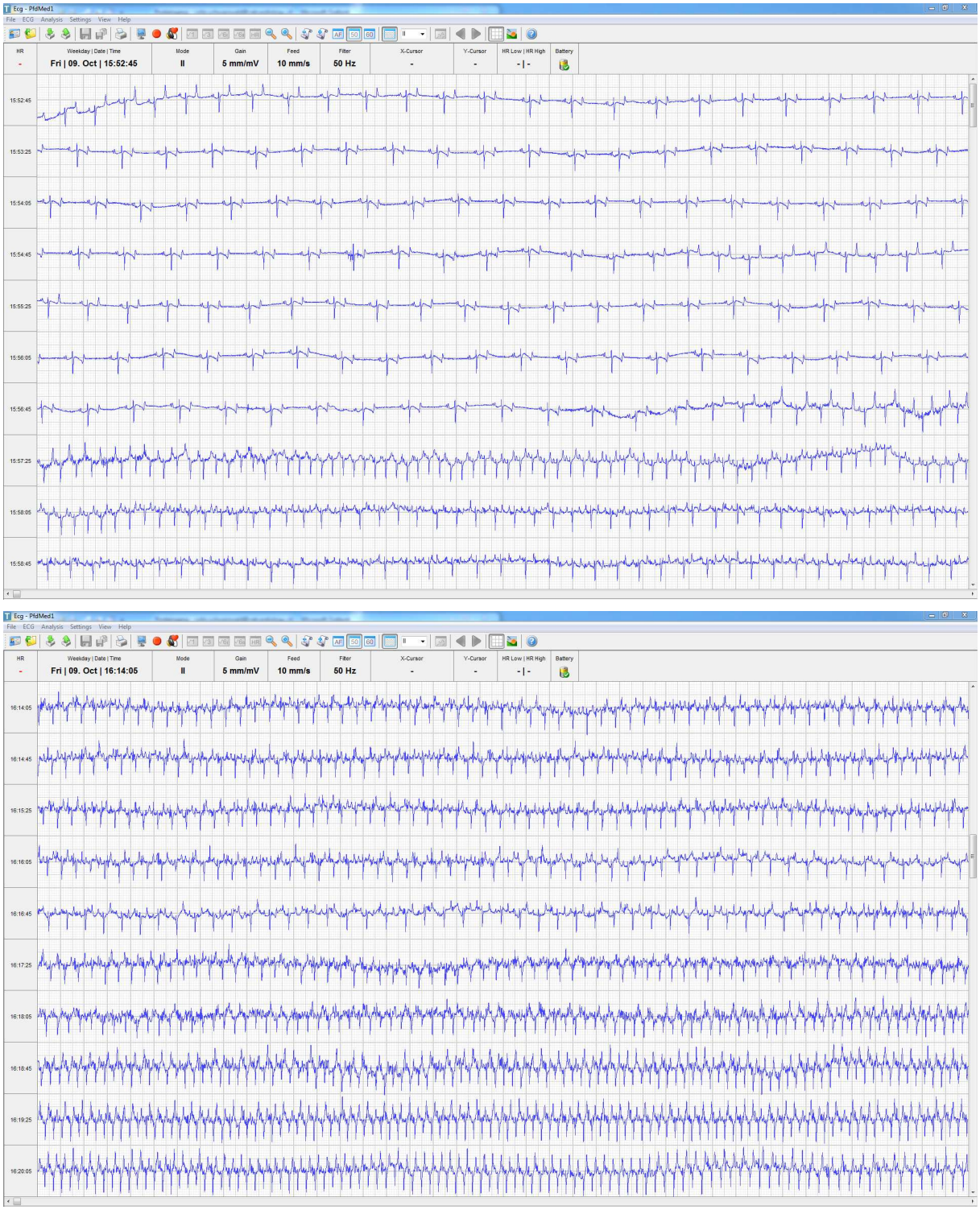


Figure 1a: Overview screen (lead II) showing segments of an exercising ECG recorded on a healthy horse undergoing a standardized treadmill exercise test. The overview shows different segments recorded at different gaits and heart rates. Overall, the quality of the recording appears sufficient, although it is evident that recordings at higher heart rates and faster gaits contain some motion artefacts.

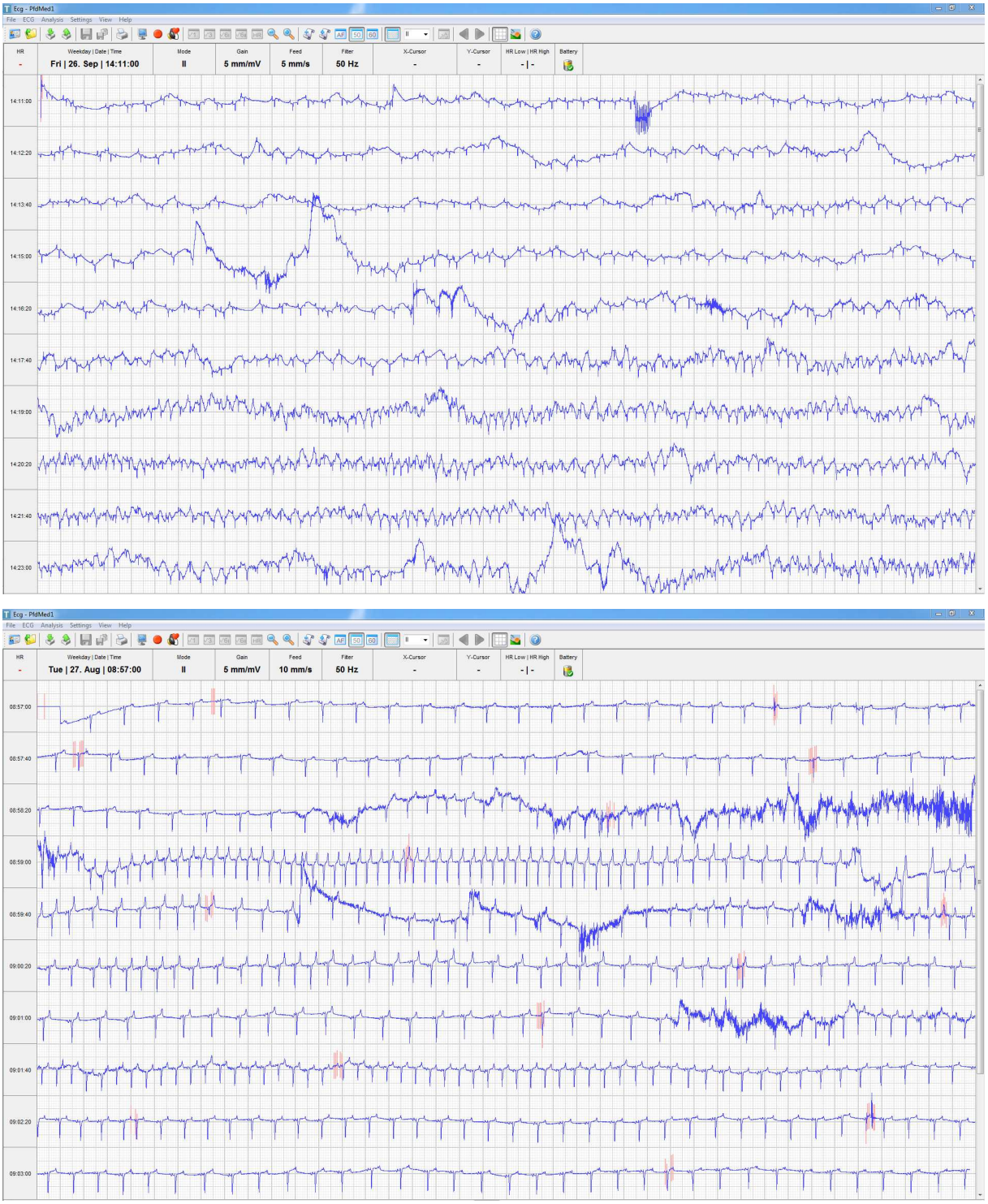


Figure 1b: Overview screen (lead II) showing segments of exercising ECGs of two horses at different gaits and heart rates. The quality of the recordings is poor and affected by a variety of artefacts. The red shaded marks shown in the bottom example indicate loss of Bluetooth signal of the telemetric recording.

2. *Screen for obvious rhythm events*

As a next step, still using the overview screen, one should try to determine the underlying rhythm (i.e., usually a sinus rhythm or atrial fibrillation) and visually screen for obvious physiologic or pathologic rhythm events, in order to get a first impression on cardiac rhythm during all phases of the exercise test. Some rhythm events such as physiologic AV blocks or pathologic ventricular ectopic beats might show up as obvious events interrupting the regular ECG pattern (**Figure 2a**), allowing an immediate rhythm diagnosis. Paper speed and gain can be adjusted as necessary to facilitate pattern reading.

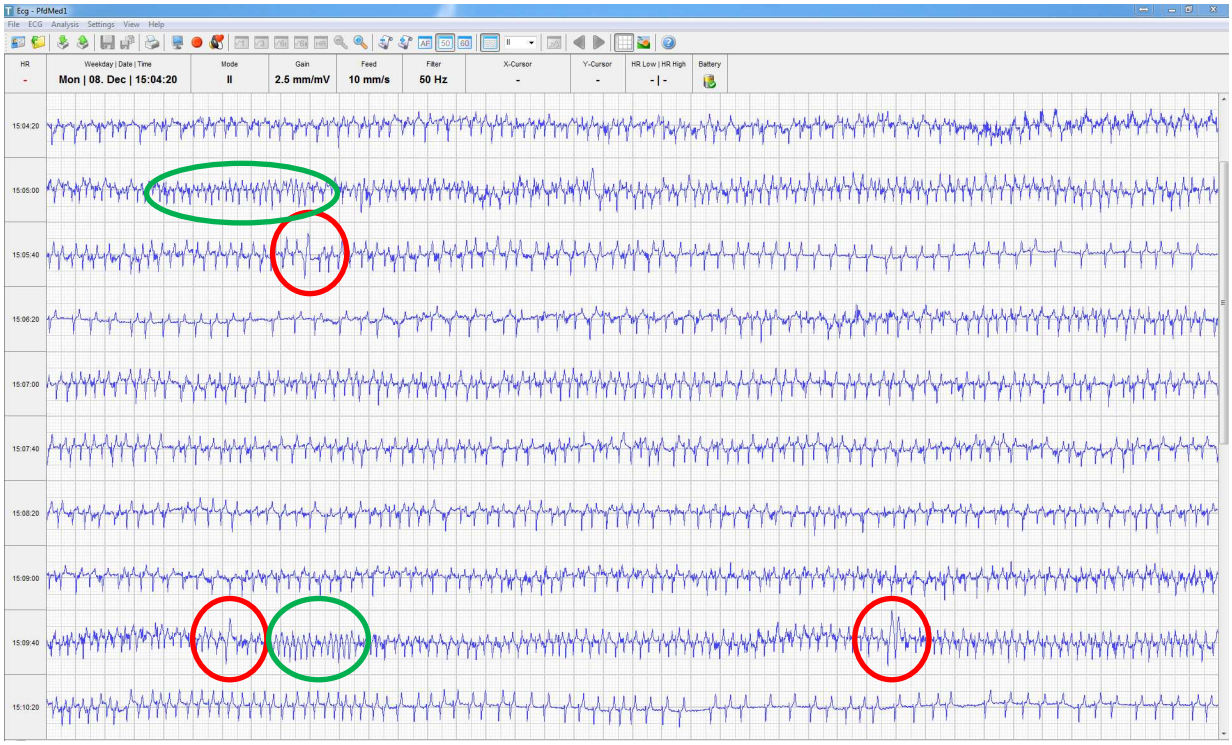


Figure 2a: Overview screen of an exercising ECG recorded in a horse with atrial fibrillation. The baseline rhythm is irregularly irregular, most obvious during periods with lower heart rates. Abnormal QRS-T complexes, presumably premature ventricular complexes (PVCs) occurring as two single events and as a couplet with short coupling interval, are evident (red circles). Also, two episodes of a subjectively more regular and more rapid rhythm can be seen (green circles), suggesting either accelerated atrio-ventricular (AV) conduction or a short run of ventricular tachycardia.

2.1. Details

If obvious rhythm events are detected on the overview screen, double clicking on the respective event will switch to the detail screen, allowing more detailed assessment (**Figure 2b**). However, subtle abnormalities such as premature atrial complexes with long coupling intervals can be easily missed on the overview screen and pure visual screening is not sensitive enough to detect all abnormal rhythm events.

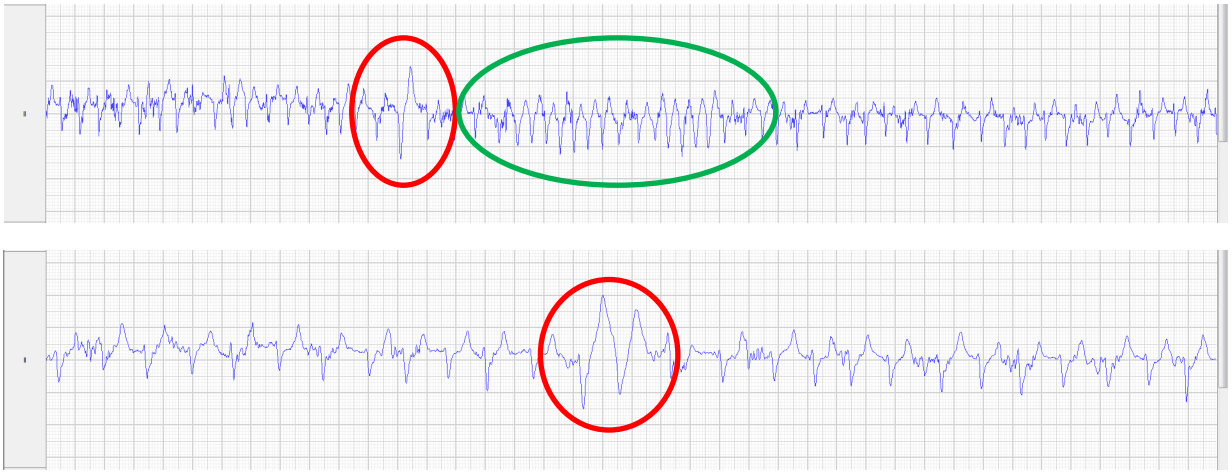


Figure 2b: Detail views of three of the rhythm events detected in the overview screen (**Figure 2a**). The top ECG segment shows an abnormally shaped, wide QRS-T complex suggesting a PVC (red circle), followed by an episode of a subjectively more regular and more rapid rhythm (green circle) suggesting either accelerated AV conduction or a short run of ventricular tachycardia. The bottom ECG segment shows two abnormally shaped, wide QRS-T complexes suggesting a ventricular couplet with a very short coupling interval (near R-on-T; red circle). Note that paper speed differs between the two segments.

3. RR interval analysis

Detection of premature beats can be difficult at high heart rates during exercise, because differences in RR intervals (*) can be very subtle and visual pattern reading might be impaired by motion artefacts (**Figure 3a).**
(* Note that the prominent negative deflection of the QRS complex in horses depicts the S wave; nonetheless, the terms R wave and RR intervals are used here to comply with the terminology used in the analysis software.)

Figure 3a: Exercising ECG at a heart rate of approximately 88 /min. Some variation in cycle length is present, but difficult to identify by simple visual inspection and pattern reading. Differences in RR intervals are subtle ("normal" RR intervals marked by red arrows, shorter RR interval marked by green arrow) and blurred by the varying electrical baseline.

Therefore, quantitative RR interval analysis is crucial for assessment of exercising ECGs. On contemporary ECG analysis systems this can be achieved using automated computer algorithms that are able to detect R waves on digitally recorded ECG tracings. The ECG analysis function of the Televet software not only allows automated RR detection but also provides the possibility to mark unusually short or long cardiac cycles based on the % deviation of consecutive RR intervals. In our cardiology service we currently use cut-off values of 20% maximum deviation of consecutive RR intervals for ECG analyses at rest and 8% for ECGs recorded during exercise, respectively (**Figure 3b**, top). In other words, QRS complexes occurring at a cycle length (i.e. RR interval) that is > 20% (at rest) or > 8%

(during exercise) shorter or longer than the preceding cycle length will be marked in red as “premature” or “delayed” beats (**Figure 3b**, bottom). However, these cut-offs are largely empiric and the distinction between (physiologic) heart rate variability and (pathologic) prematurity can be difficult. The current knowledge indicates that during sinus rhythm a sudden shortening of the RR interval by more than 15-20% at rest and 5-8% during trot and canter phases indicates prematurity, whereas variation between consecutive RR intervals of less than 15% and 5%, respectively, likely indicates normal (sino-atrial) heart rate variability, particularly when variation is gradually distributed over a period of multiple beats. However, exact criteria are lacking to date.

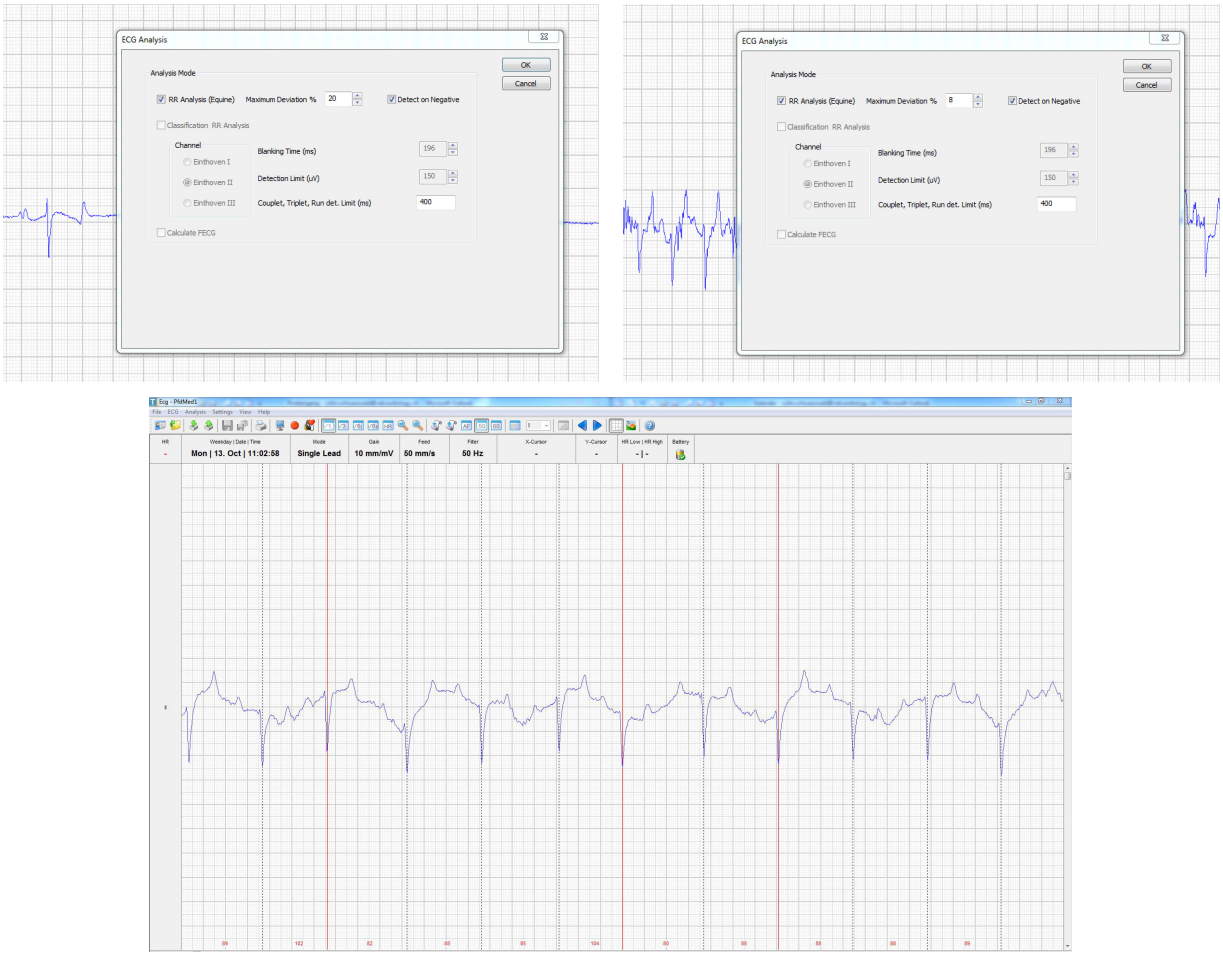


Figure 3b: Top – ECG Analysis function of the Televet software. „RR Analysis (Equine)“ is chosen and a „Maximum Deviation %“ is selected (e.g., 20% for resting ECGs and 8% for exercise ECGs). „Detect on negative“ should be checked if negative S waves are predominant (which is usually the case in equine stress ECGs, depending on electrode placement“. Note that the option “Classification RR Analysis” (which is based on P-QRS-T templates) is not intended for use in horses, since P-QRS-T conformation is too variable, particularly for stress ECGs. Bottom – Exercising ECG shown in Figure 3a after automated RR Analysis using a cut-off for maximum RR deviation of 8%. The QRS complexes (i.e., S waves) are marked by dotted lines if the consecutive RR intervals vary less than the given maximum % deviation or by a red line if the respective RR interval varies more than the given cut-off compared to the preceding RR interval. The instantaneous heart rate is displayed for each cardiac cycle at the bottom of the screen.

It is important to realize that beats that are marked in red by the software (based on the operator-defined cut-off for maximum % deviation of consecutive RR intervals) should not automatically imply that these are truly abnormal (pathologic) beats. Similarly, beats that are not marked by the software are not necessarily normal beats. For example, sometimes a slightly premature beat might be marked with a "normal" dotted line but the normal sinus beat following the resulting pause will be marked in red. With physiologic 2nd degree AV block, the QRS complex following the block will be marked red, and depending on the cut-off chosen for maximum % deviation, sinus arrhythmia may cause the software to mark irregular beats (**Figure 3c**). By choosing different cut-offs for the maximum % deviation of consecutive RR intervals, the operator can modify the sensitivity and specificity of the automated algorithm to detect premature or delayed beats. By decreasing the cut-off, the system will become more sensitive but less specific to detect premature or delayed beats. Increasing the cut-off will have the opposite effect (**Figure 3d**). For horses in atrial fibrillation, larger detection limits can be used to avoid triggering too many irregular beats.

Figure 3c: Examples of normal sinus beats marked after a 2nd degree AV block (top) and during sinus arrhythmia (bottom).

Figure 3d: Effect of different cut-offs chosen for maximum RR deviation. This example shows that decreasing the cut-off will increase the system's sensitivity to detect premature (or delayed) beats. While no beat is labeled "premature" using a cut-off of 30% (top left), one, three and four beats, respectively, are labeled "premature" when using lower cut-offs of 20, 8 and 5%. This demonstrates that differentiation between physiologic sinus arrhythmia and pathologic premature beats can be difficult and should not be based solely on automated RR analysis.

Finally, the automated computer algorithms are imperfect and prone to error. Beats can be falsely marked by the software because of RR detection errors (**Figure 3e**). Therefore, visual validation of correct R wave detection by the

operator is mandatory, particularly at faster gaits when ECG tracings are affected by motion artefacts. In horses with an underlying normal sinus rhythm and adequate recording quality, the verification process can initially be focused on marked beats and the adjacent ECG segments, realizing that some detection errors might go undetected. However, in most cases, page by page full disclosure assessment is necessary for exercising ECGs that are affected by motion artefacts. Manual correction of falsely marked or missed beats by the operator is possible in the Televet software (**Figure 3e**). Remember that normal (sinus) QRS complexes are the narrowest possible complexes that can occur physiologically. They are always followed by a T wave. Therefore, wave deflections that are narrower than the narrowest, presumably normal QRS complex and those that are not followed by a T wave must be considered artefacts.

Figure 3e: Left row – Erroneous detection of QRS complexes by the automated RR analysis algorithm, causing some complexes to be falsely marked in red. Right row – Same ECG segments after manual correction by Ctrl & left-click onto the respective mark (to remove it) or QRS complex (to mark it). Note that not all erroneous detections will result in a red mark; if the deviation is small, complexes will be marked with a regular dotted line (last example). Therefore, visual verification of RR tracking is critical if subtle abnormalities are to be detected.

4. *ECG analysis*

4.1. Heart rate (HR)

After RR analysis, the Televet software allows to display the course of the heart rate over time (HR view). This is helpful to obtain an overview on maximum exercising HR (**Figure 4a**). It is however important to note that in the current software version (Televet v. 6.0), RR intervals that are marked red (i.e. those that differ more than the cut-off for % RR deviation set for the analysis) are not displayed in the HR view (personal communication, Klaus Engel, Engel Engineering Services GmbH). Therefore, this graph cannot be used to assess the quality of analysis (i.e. estimate the number of erroneously detected QRS complexes) or the frequency and temporal distribution of premature beats. Another limitation of the HR view is that the time (x) axis can only be scaled to a certain extent, so that the course of the HR over time of long-term recordings cannot be displayed on one single screen (this limitation however mostly applies to long-term Holter recordings, not to shorter exercising ECGs). Another problem, which is more critical for exercising ECGs, is that the y axis is limited to a maximum heart rate of 240 /min.

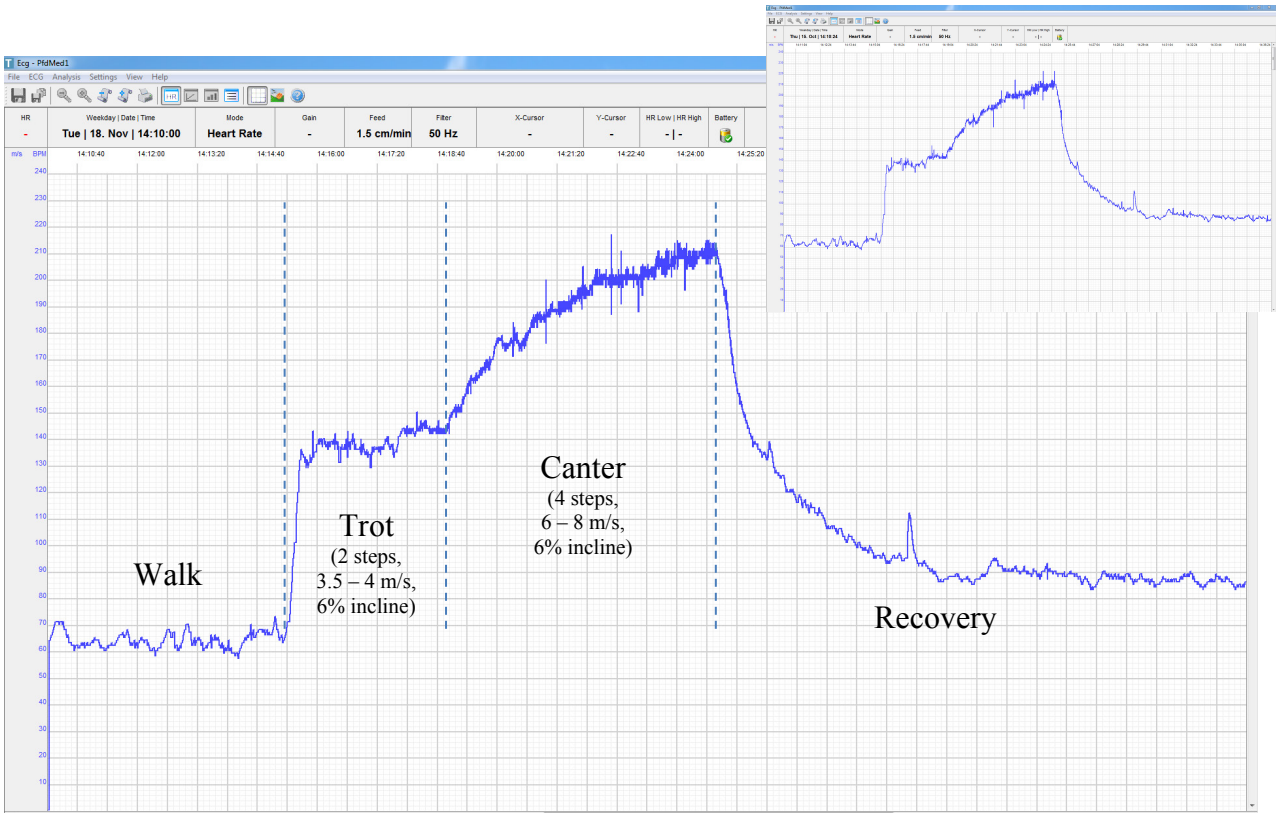


Figure 4a: Example of an ECG recorded during a high-speed treadmill exercise examination. The heart rate (HR) view in the Televet software provides an overview on the course of the HR over time. The different phases of exercise (i.e. walk, trot, canter, recovery) can be identified. The current version of the software (Televet v6.0) introduces some bias in that complexes marked in red (i.e. those that differ more than the cut-off for % RR deviation set for the analysis), including AV blocks, premature beats, and (if present) artefacts are not displayed in the HR view. Therefore, visual verification of RR tracking and manual correction, as described above, does not markedly influence the HR view. In this example, the large graph is based on the corrected RR time series, whereas the insertion in the right top corner is based on the uncorrected RR time series.

4.2. Rhythm analysis

In horses with an underlying normal sinus rhythm, adequate ECG recording quality and accurate RR analysis using appropriate cut-off values for maximum % RR deviation, rhythm analysis can initially be limited to marked beats and the adjacent ECG segments. However, some rhythm disturbances might be missed this way and page-by-page visual inspection of the entire ECG recording is usually necessary. In particular, full disclosure assessment is mandatory if the underlying rhythm is atrial fibrillation (AF), since the automated algorithm will detect marked arrhythmia (as expected) but cannot differentiate between very short cycles due to AF with rapid ventricular response and those due to premature ventricular complexes or ventricular tachycardia superimposed to AF.

Rhythm analysis is generally based on identification of P waves and QRS-T complexes and characterization of their shape and their temporal relationship to each other. A normal sinus rhythm during is characterized by a regular rhythm with QRS complexes of uniform configuration. There should be a P wave for every QRS-T complex and conversely a QRS-T complex for every P wave. However, P waves become difficult to detect during exercise, because at higher HR they become superimposed to the preceding T waves. Furthermore, motion artefacts decrease the signal-to-noise ratio, so that the small P waves may not be differentiated from baseline noise. Therefore, rhythm analysis on exercising ECGs is largely based on assessment of RR intervals (i.e. regular, regularly irregular, irregularly irregular; premature complexes, pauses) and QRS-T configuration. The QRS-T configuration is very variable in the horse, particularly during exercise at higher heart rates. Exercise causes quite dramatic (physiologic) alterations in T wave morphology. Deviation of the ST segment and increased amplitude of the T wave are expected even in normal horses during and after exercise.

Variability over time of the frequency and "malignancy" of atrial and ventricular arrhythmias in horses is currently unknown. Also, the influence of different types of exercise (e.g., lunging vs. treadmill exercise vs. ridden exercise) on ventricular arrhythmias is not well defined.

4.2.1. Transient post-exercise vagal rhythms *(see case example 2 below)*

Marked sinus arrhythmia and occasionally atrio-ventricular (AV) and sino-atrial (SA) pauses can be encountered immediately following exercise in the early recovery period. These rhythms are generally attributed to a phase of autonomic (vago-sympathetic) imbalance. These arrhythmias are not considered clinically relevant, as long as they are not observed during the exercise period itself.

4.2.2. Premature atrial complexes (PACs) *(see case examples 3 below)*

Premature atrial complexes are characterized by a premature P wave (often referred to as P') that occurs earlier than expected based on the PP interval of the underlying rhythm. The P' wave often differs from the normal P wave in size and morphology. It is followed by a normal QRS-T complex.
Unfortunately, several exceptions to these characteristics of PACs render their diagnosis very challenging, even when P waves are clearly visible during exercise and in the recovery phase. Normal sinus P waves and premature P' waves can in fact be very similar in shape and size. Also, the normal sinus P waves are not static. They shorten and become more peaked during tachycardia and their morphology can change with alterations in autonomic input during sinus arrhythmia (wandering pacemaker). Therefore, differentiation between sinus arrhythmia and PACs can be difficult. Single, isolated premature beats are

more likely to be PACs, whereas PP intervals varying in a cyclic manner are more likely to represent normal variability of sinus rate (which however should not be very pronounced at high heart rates).
A PAC usually also depolarizes the sinoatrial node. The latter will then be "reset" and subsequently resumes its pacemaker activity earlier than expected based on the underlying PP rhythm. This is commonly termed "non-compensatory (incomplete) pause". However, PACs may not always reset the SA node and disturb the underlying rhythm. Generally, PACs are challenging to detect at high HR because they are not usually followed by an obvious pause.
If the impulse occurs early in diastole and arrives at the AV node before it has completely repolarized, the premature P' wave will not be conducted and will not be followed by a QRS-T complex. Premature atrial impulses can further be conducted slowly across the AV node (with first-degree AV block) or aberrantly through the ventricle as a result of incomplete repolarization or persistent refractoriness of AV or ventricular conducting tissues. Abnormal (aberrant) ventricular conduction of an atrial premature complex causes the QRS-T complex to be wider than normal or atypical in configuration. This should not be misinterpreted as a premature ventricular beat.

Isolated PACs are frequently encountered in horses. Their clinical relevance is not always clear. Occasional isolated PACs are usually well tolerated and are sometimes considered a normal variation if they occur infrequently (e.g., less than one premature beat per hour) or only during recovery from exercise. In many cases, PACs detected at rest will be suppressed by increasing sinus rate during exercise. Nonetheless, one should keep in mind that the frequent and/or longstanding occurrence of PACs may precede the development of more serious atrial arrhythmias such as atrial fibrillation.

4.2.3. Atrial flutter / fibrillation (AF) *(see case examples 4 below)*

Atrial flutter and atrial fibrillation are characterized by an irregularly irregular R-R interval with normal QRS morphology, the absence of P waves and the presence of flutter waves (termed "F" or "f"). Flutter waves resemble saw-toothed P waves without an isoelectric shelf and with a regular atrial rate of about 170-275/min. Fibrillation waves are less organized and faster (275-500/minute on intracardiac electrograms). AV conduction is variable, resulting in an irregular ventricular rate response. Atrial flutter may cause a more rapid ventricular response rate during exercise compared to atrial fibrillation. At very high heart rates during exercise, when the diastolic interval is very short and the ECG tracing affected by motion artefacts, the "f" waves can usually not be seen on the ECG tracing. And although the ventricular response becomes more regular at higher HR, the rhythm always remains irregular. This can be revealed by careful inspection of the ECG and measurement of consecutive RR intervals. Graphical display of RR and HR time series (see below) can aid in the detection of an irregular rhythm at high heart rates. A sudden onset of an irregularly irregular rhythm during exercise in a horse with a previously regular rhythm suggests onset of paroxysmal AF.

Rapid or repetitive atrial arrhythmias such as atrial flutter and atrial fibrillation (AF) are most likely to decrease cardiac output at high heart rates and are considered hemodynamically important. Atrial fibrillation is the most common arrhythmia affecting performance. Although uncommon, collapse during exercise has been reported with AF. Safety is a particular concern with persistent AF when the average maximal heart rate (HR) during exercise at an intensity that is at or slightly exceeding the horse's normal

activities is greater than 220/minute. Additionally, ventricular ectopy during exercise or during sympathetic stimulation is not unfrequently observed in association with AF and indicates a possible risk for SCD, particularly when short R-R intervals or R-on-T phenomenon are present. AF associated with exercise-induced ventricular arrhythmias resulting in SCD has been documented by telemetric ECG in at least one horse.

4.2.4. Premature ventricular complexes (PVCs) *(see case examples 5 below)*

Complexes of ventricular origin are conducted abnormally and more slowly, resulting in a widened QRS, an abnormal QRS orientation, and abnormal T waves. Hence, on ECG examination, they will have a wide and bizarre configuration. However, the difference between normal QRS complexes and PVCs may become less obvious during exercise, since a narrowing of all QRS complexes occurs at higher heart rates and because motion artefacts may affect the QRS-T configuration of normal and abnormal beats. Also, PVCs originating from high in the His-Purkinje system or the AV junction result in a narrow, relatively normal-appearing QRS complex with normal initial activation and electrical axis. Often times, they appear somewhat taller than the normal QRS complexes.
Usually, PVCs are not retrogradely conducted to the atria and will not disturb sinoatrial nodal function. Therefore, PVCs break the normal cardiac rhythm with the normal sinus P wave still present (but not associated with the PVCs). The respective P wave cannot elicit a ventricular response, because the PVC has turned the ventricle refractory. Consequently and in contrast to PACs, the rhythm of the sinus node is not disrupted and the sinus rhythm (i.e. PP intervals) remains constant despite the PVC. This results in a relatively long pause after the premature beat, which termed “compensatory (complete) pause” and can usually be detected during ECG analysis. However, similar as for PACs, on rare occasions PVCs can be interpolated, in which case they do not result in a compensatory pause.
The overall consequence is that the traditional criteria to differentiate ventricular from supraventricular arrhythmias (association with P wave, shape and orientation of the QRS-T complex, non-compensatory vs. compensatory pauses) are difficult to apply to exercising ECGs and at high heart rates, and that often times premature ectopic beats cannot be unambiguously classified as supraventricular and ventricular, respectively.

Premature complexes may also appear as couplets (pairs), triplets, or short runs. Repetitive ectopic complexes that occur in short bursts or runs are termed nonsustained or paroxysmal ventricular tachycardias. Sustained ventricular tachycardias may also occur. With sustained ventricular tachycardias, P waves may be identified but are not conducted and therefore not consistently associated with a QRS complex. Some P waves may be buried in the ectopic QRS-T complexes (especially at higher rates of ventricular activation), making their identification difficult. The use of ECG calipers helps determining the P-P interval and can facilitate the identification of P waves. Ventricular tachycardias are referred to as uniform (monomorphic) if the QRS-T morphology of the ectopic beats is consistent throughout the recording, and as multiform (polymorphic) if two or more abnormal QRS-T configurations can be identified.

Premature ventricular complexes are usually considered abnormal in the horse, although isolated ventricular ectopic complexes are not unfrequently encountered in supposedly healthy during and after

exercise. Conversely, in some horses with PVCs observed at rest, the ectopic focus is overdriven by the increasing sinus rate during exercise. Generally, the clinical relevance of an occasional premature ventricular complex in the horse is difficult to ascertain. The complexity of ventricular arrhythmias is presumed to relate to the risk of hypotension and sudden cardiac death (SCD) because of ventricular fibrillation. However, risk stratification for ventricular arrhythmias is imperfect, particularly in horses with isolated PVCs.

In the absence of clear evidence, recommendations should be biased toward safety, as opposed to maintaining athletic activity. Certainly, a history of collapse or co-existence of important structural heart disease (and cardiomegaly) raises great concern in a horse with PVCs. However, in the absence of obvious clinical signs or of serious structural heart disease, the risk of ventricular ectopy is usually defined by electrocardiographic characteristics, accepting the limitations of this analysis. This assessment includes timing, rate and morphology of the ectopic activity.

As a general rule, ventricular arrhythmias should be considered complex or "malignant" and potentially life threatening, if they are characterized by one or more of the following criteria:

- Repetitive or sustained ectopic rhythms
- Very rapid ventricular rate
- Multiform or polymorphic QRS morphology (including torsades de pointes)
- Short coupling interval with R-on-T phenomenon (i.e., PVCs occurring on the peak of the preceding T wave)

Complex ventricular arrhythmias can induce hemodynamic impairment resulting in clinical evidence of low cardiac output (e.g., weakness, stumbling, pale mucous membranes, prolonged CRT, syncope) and hypotension. Electrical instability is a particular concern in malignant ventricular arrhythmias and can cause ventricular tachycardia to progress into ventricular flutter or ventricular fibrillation. These commonly represent terminal events.

5. Advanced analyses of RR time series using graphical and statistical methods

The RR time series generated by the Televet system can be exported to be used for advanced statistical and graphical analyses. This can be helpful to (1) better quantify heart rate, (2) detect rhythm disturbances, artefacts and outliers that were previously missed, and (3) get a better overview on frequency and temporal distribution of arrhythmias. However, these methods cannot be used to differentiate between supraventricular and ventricular arrhythmias.

5.1. RR export

After automated RR analysis and visual verification by the operator, RR time series can be exported. Using the "File/Export RR Intervals…/RR-Intervals only" option in Televet *, a text file will be created with all consecutive RR intervals (in milliseconds, ms) listed in a column. These can then be imported or copied/pasted in any graphical or statistical software. The corresponding instantaneous HR can be calculated (e.g. in MS Excel or in GraphPad PRISM) using the following equation: HR = 60'000 / RR. (* The option "File/Export RR Intervals…/RR-Intervals and additional data" includes all consecutive RR intervals and the corresponding time stamp and heart rate. The RR intervals labeled as abnormal (red) will be listed as negative numbers; therefore

absolute values need to be calculated before the data can be used for graphical or statistical analyses. The time stamp aids in identifying individual RR intervals if necessary.)

5.2. Graphical display of RR and HR time series

The RR and HR time series can be plotted over time (i.e. cycle by cycle) using 2D line graphs in MS Excel or Kubios HRV software (Figures 5a and 5c). These graphs provide more detail than the standard Televet HR plots (Figure 4a) because all consecutive RR intervals, as marked by the software and independent on whether they are marked with black dotted lines or with red lines (i.e. independent of the % deviation of RR intervals), are considered for graphical display. Besides graphical assessment of RR and HR during the different phases of exercise, the graphs allow simple visual quality assessment of an RR time series. When applied to manually corrected, verified RR time series, these plots can aid in the detection of rhythm disturbances and the assessment of frequency and temporal distribution of arrhythmias (see examples below). The Kubios HRV software offers the advantage that different segments of a RR time series, e.g. corresponding to the different phases of an exercise test, can easily be selected and graphically analyzed (Figure 5d).
Additionally, so called Poincaré plots can be generated as scatter plots in which each RR interval (RR) is plotted against the next following RR interval (RR+1) (Figures 5a and 5d). These plots allow simple visual assessment of variability of consecutive RR intervals and can further aid in the detection of ectopic beats (see examples below).
Sometimes, RR and HR time series and Poincaré plots will result in identification of rhythm disturbances that remained undetected during routine visual assessment. This should prompt the clinician to return to the full disclosure view of the ECG analysis software to look at the individual complexes and identify the cause of such rhythm disturbances.

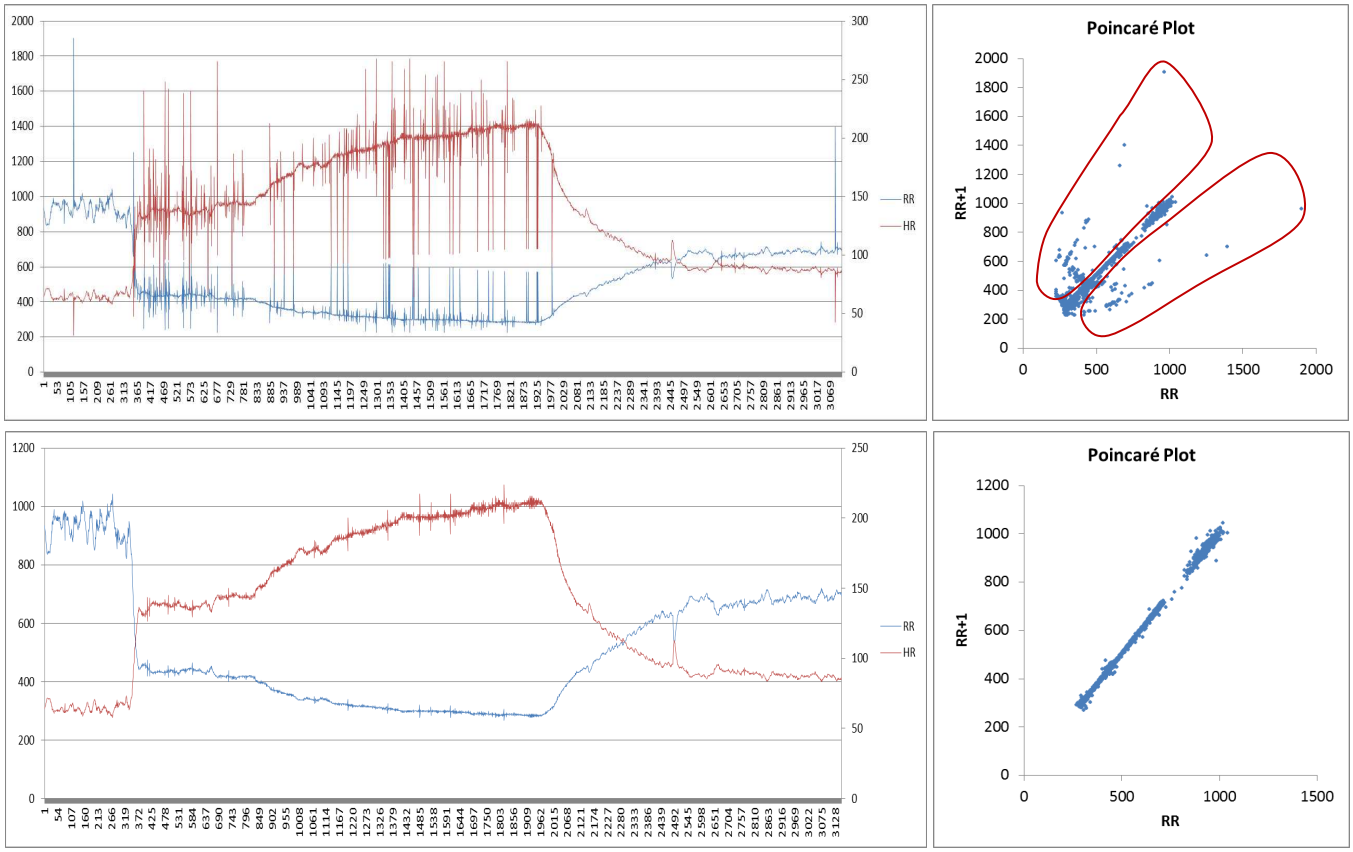


Figure 5a: RR and HR time series displayed as 2D line plots (left) and Poincaré plots (right) generated in MS Excel using the same dataset shown in Figure 4a. As opposed to the Televet HR plot, these plots include all consecutive RR intervals (independent of % RR deviation) and therefore provide more detail. The top graphs are based on the RR time series exported from the uncorrected ECG. The large positive and negative spikes represent artefacts caused by erroneous detection of QRS complexes by the automated RR analysis algorithm (Figure 3e). These artefacts are also evident in the Poincaré plot (red circles). The bottom graphs are based on the same ECG after manual correction and verification of correct R wave detection by the operator. Some smaller spikes are still present. They either represent remaining artefacts (erroneously detected beats that were missed during the manual correction and verification process) or true rhythm disturbances (e.g., premature beats followed by pauses). Similar spikes are also seen in Figure 4a. The Poincaré plot indicates that variability between RR intervals is overall low, hence consecutive RR intervals are of similar length at all heart rates; therefore, all data points are lined up along the diagonal line of identity.

Figure 5b: If smaller spikes are still remaining after the manual correction and verification process (see Figures 4a and 5a), the respective cardiac cycles should be inspected in the detail screen. This can either be achieved by double-clicking onto the spikes in the Televet HR view (Figure 4a) or by identifying the respective RR interval using the corresponding time stamp (if available after export; see above). In this example, the spikes seen in the RR and HR time series represent artefacts due to RR detection errors that were missed during manual correction. This demonstrates that very small measurement errors can markedly influence the appearance of the RR and HR time series and result in distinct spikes at higher heart rates. It is further important to note that as of version 6.0 of the Televet software, manual correction of such minor detection errors may not be possible, since the software will not accept placing the corrected mark as necessary if the shape of the QRS complex is altered by motion artefacts.

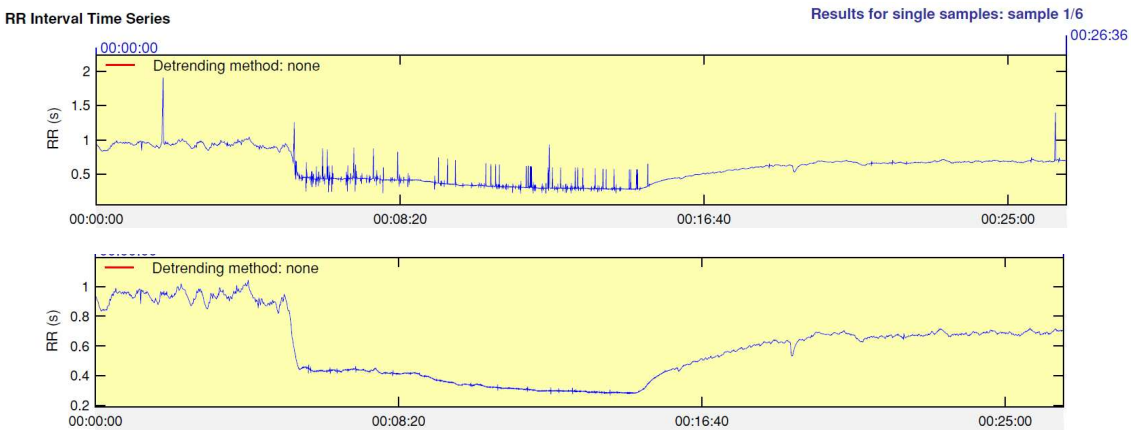


Figure 5c: Uncorrected (top) and corrected (bottom) RR time series displayed in Kubios HRV software using the same dataset as shown in Figures 4a and 5a. The RR time series corresponds to the one generated in MS Excel.

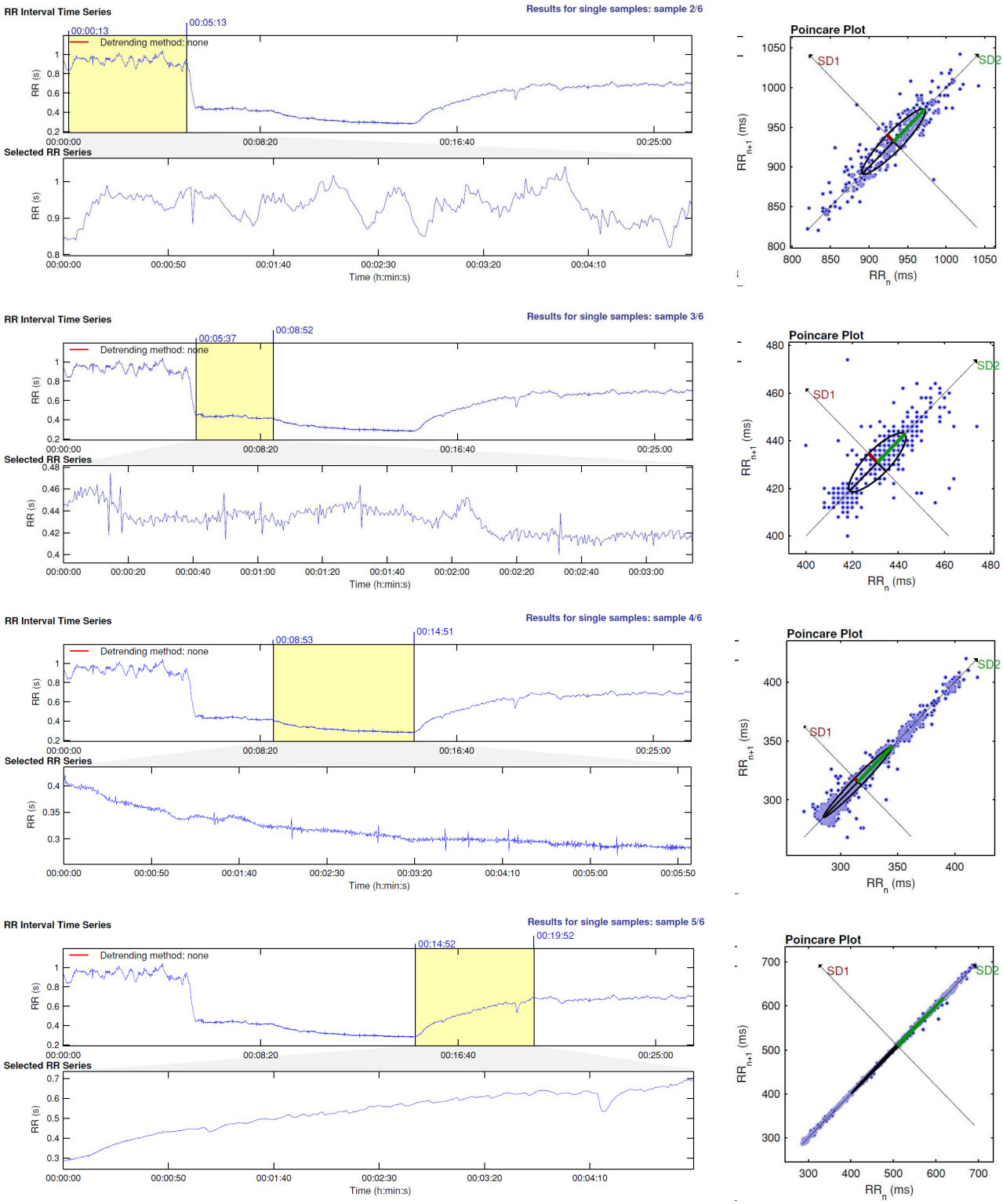


Figure 5d: RR time series (left) and Poincaré plots (right) generated in Kubios HRV software using the same dataset as shown in Figures 4a and 5a after manual correction and verification of correct R wave detection by the operator (uncorrected time series not shown). Kubios HRV does not provide HR time series. However, Kubios HRV allows simple selection of different segments of a RR time series, corresponding to the different phases of an exercise test (from top: walk, trot, canter, recovery). The spikes seen in the trot and canter phases represent artefacts (see Figure 5b). The Poincaré plots visualize the degree of beat-to-beat variability (see Figure 5a). Note that the Kubios HRV automatically scales the axes; therefore, plots representing similar variability might look different.

5.3. Advanced graphical and statistical analyses of RR and HR time series

Advanced graphical and statistical analyses of RR and HR time series may further aid in the assessment of exercising ECGs. These type of analyses are currently subject of several investigations with the goal to identify statistical parameters that allow some degree of quantification of arrhythmia and differentiation between physiologic heart rate variability and pathologic premature beats.

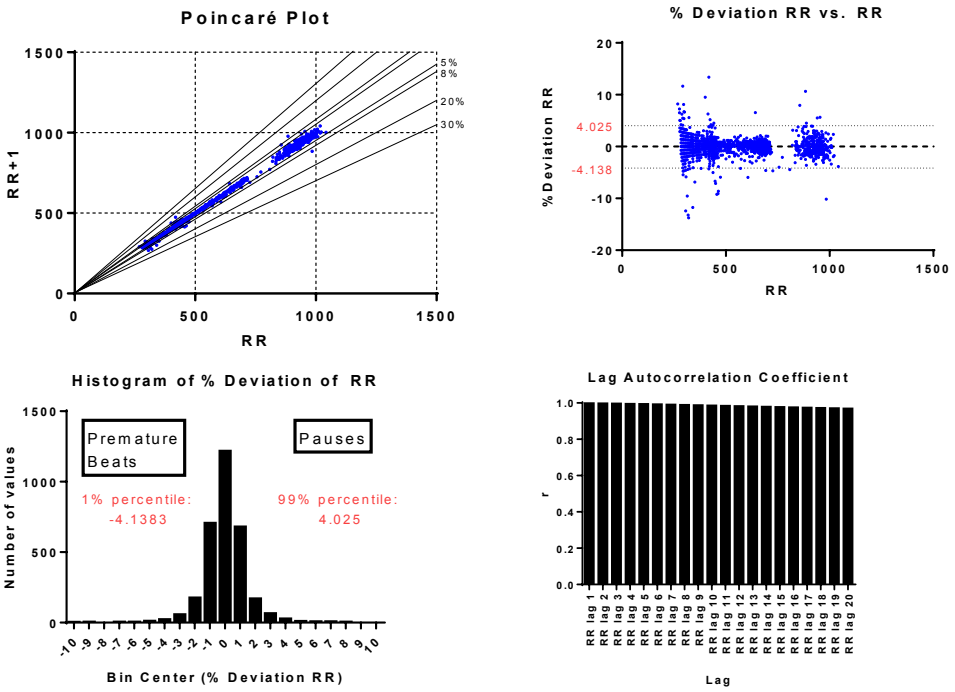
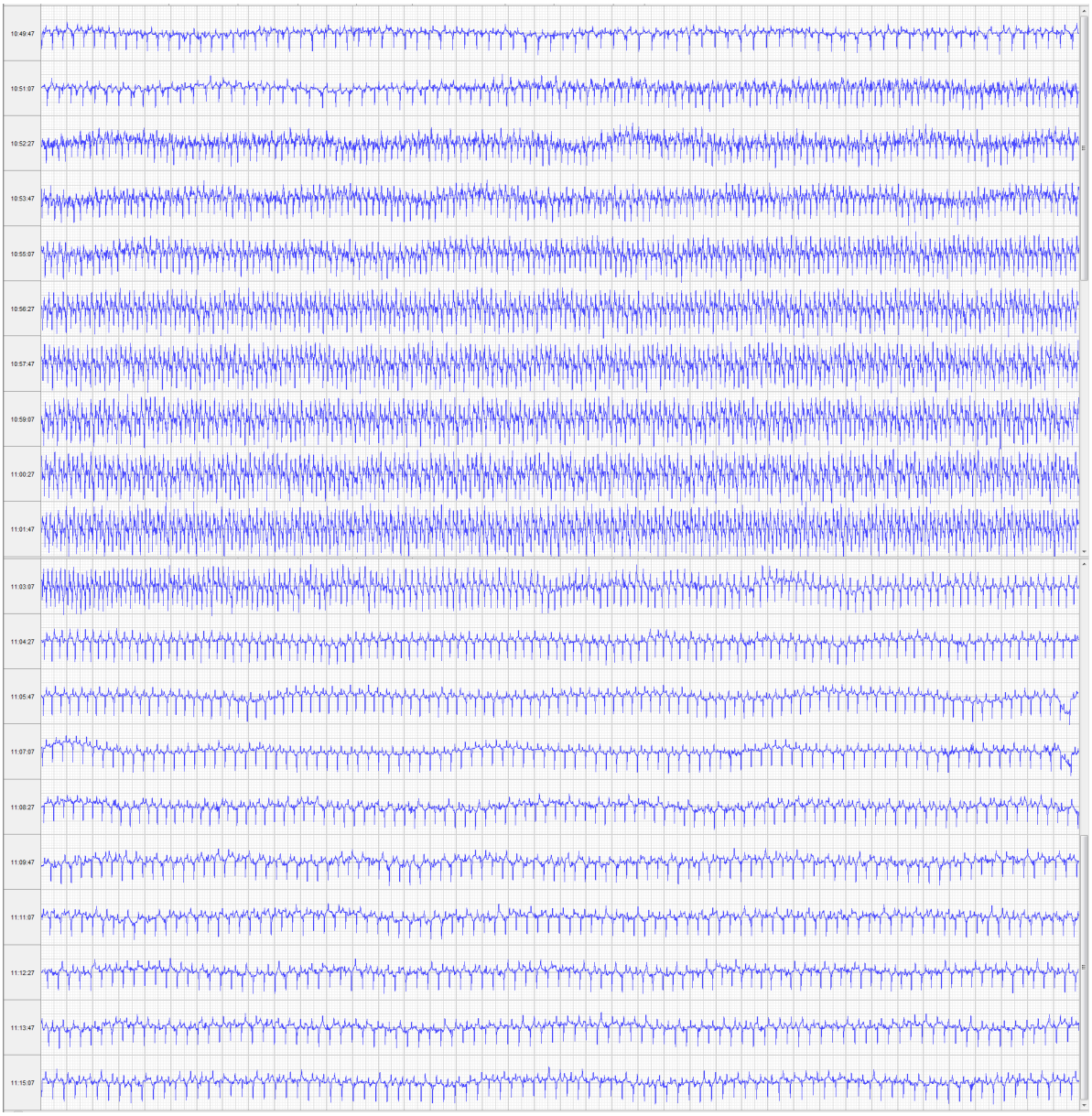


Figure 5e: Advanced graphical and statistical analysis of the RR time series shown in Figures 4a and 5a-c using GraphPad PRISM. The Poincaré plot (top left) indicates that RR variability is mostly less than 5% independent of RR. The % Deviation RR vs. RR plot (top right; the dotted lines depict the 1% and 99% percentiles) and the Histogram of % Deviation RR (bottom left) confirm this. The 1% percentile of -4.1383% indicates that in this horse only 1% of RR intervals (i.e. approx. 31 intervals) are >4.1383% shorter than the preceding RR interval. Similarly, 1% of RR intervals are >4.025% longer than the preceding RR. The Lag Autocorrelation Coefficient plot (bottom right) provides an assessment of the correlation of RR intervals with the 20 preceding RR intervals. In healthy horses in sinus rhythm, correlation should be high, as indicated by a correlation coefficient (r) of close to 1.0.

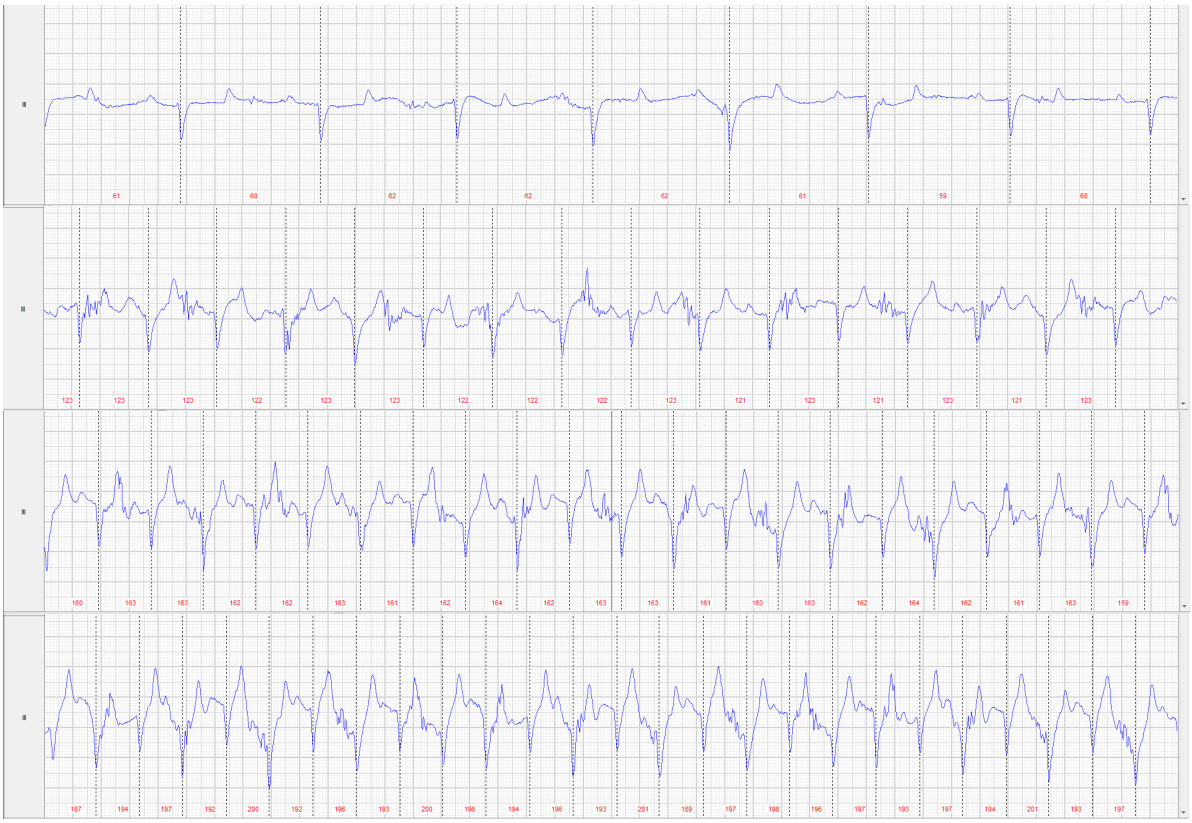
Case examples

1. Healthy horse undergoing a high-speed treadmill exercise test

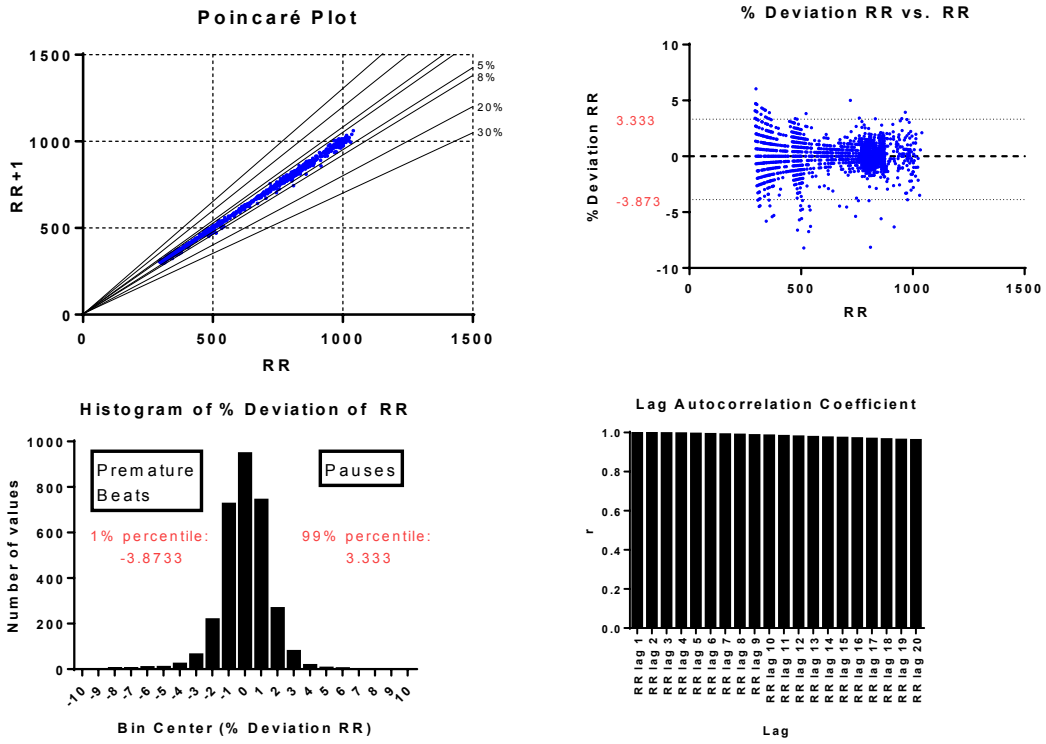


Overview screen showing a regular sinus rhythm at different heart rates and gaits.

RR and HR time series (left) and Poincaré plot (right) indicating a regular rhythm and low beat-to-beat variability.

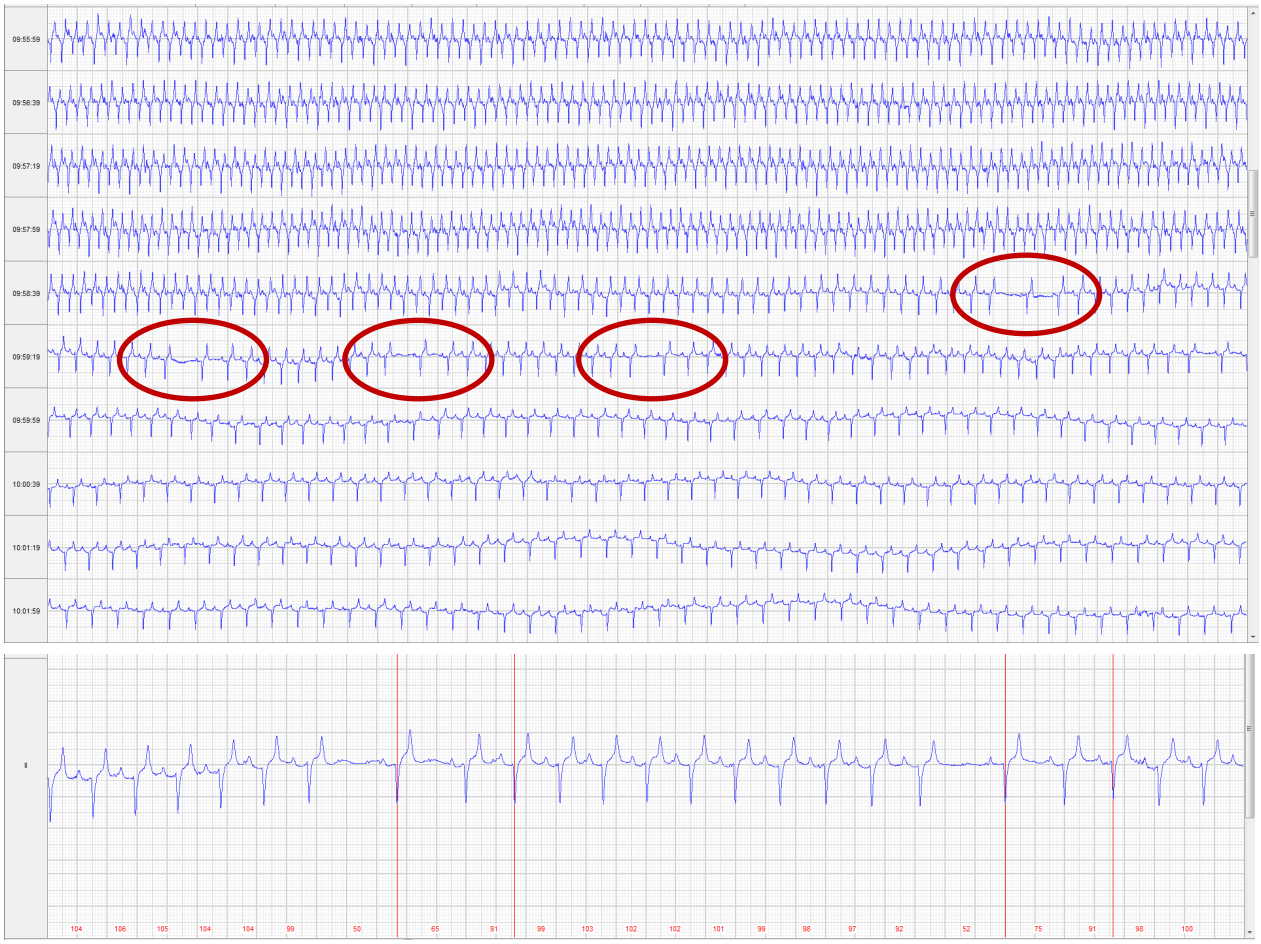


Detail view at different gaits and heart rates. From top: walk, trot, slow canter, fast canter/gallop. The P-QRS-T configuration is present at all gaits. However, at faster gaits, the P wave becomes less distinct, partly fuses with the preceding T wave and is masked by motion artefacts.

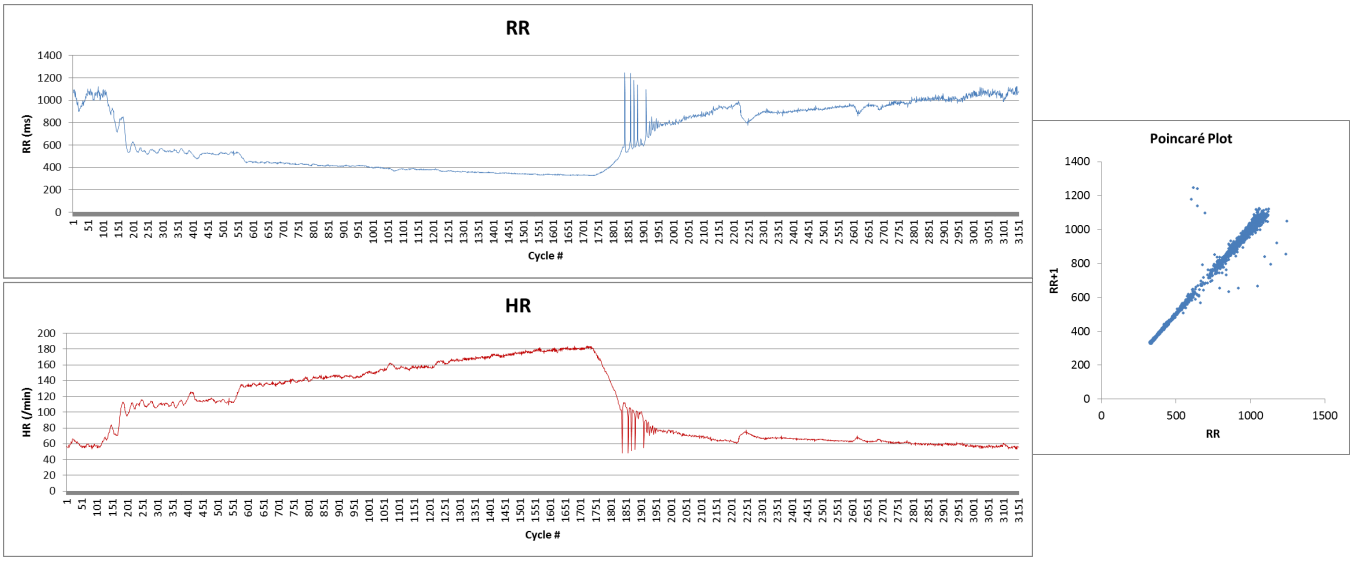


Detailed graphical and statistical analysis. All plots indicate low variability between consecutive beats. Only 1% of RR intervals (i.e., approx. 31 beats) are > 3.87 % shorter than the preceding RR. The lag autocorrelation coefficients approach 1.0 over the entire range of 1-20 beats.

2. *Vagal rhythms during recovery*



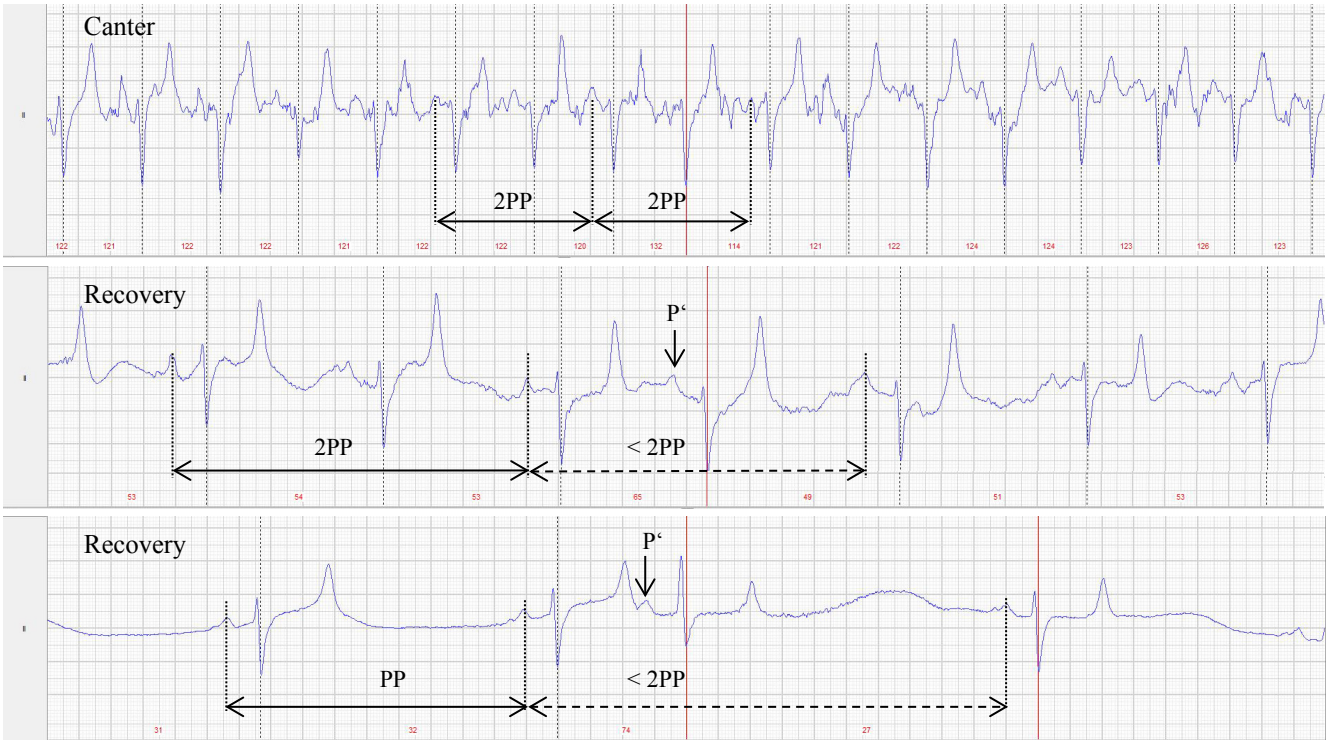
Overview screen (top) and detail view (bottom) of a horse with sinus pauses during the recovery period from exercise (red circles), occurring at a heart rate of approximately 100 beats/min. This is considered physiologic.



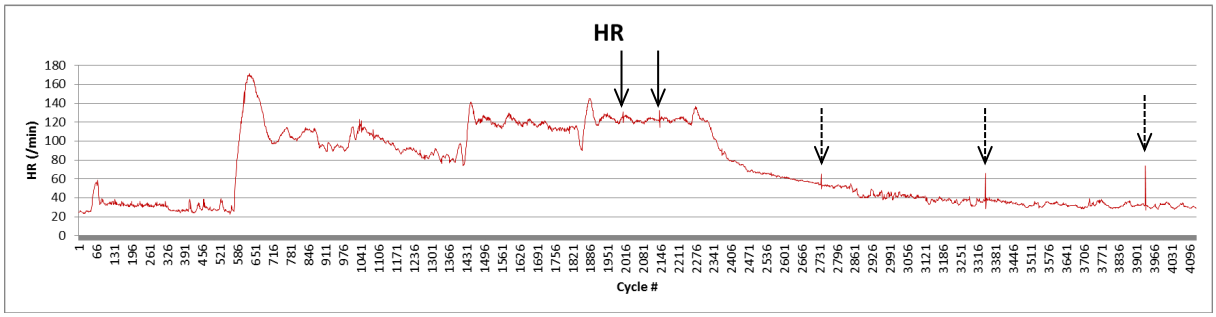
RR and HR time series and Poincaré plot. The plots indicate a regular rhythm during walk, trot and canter phases. However, a series of sharp spikes is evident during the recovery period, corresponding to the sinus pauses. In the Poincaré plot, the sinus pauses show up as a cloud of individual data points remote from the line of identity.

3. Premature atrial complexes

3.1. Occasional single PACs during work and recovery

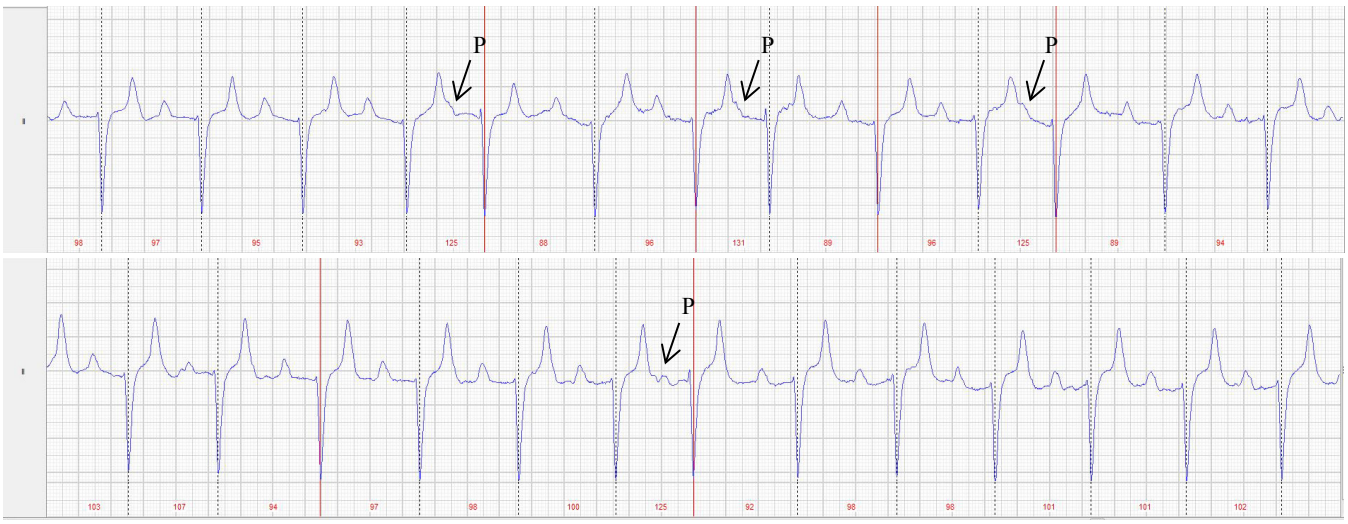


ECG at a canter and during the recovery from lunging exercise. At high heart rates during the canter phase (top), premature complexes are difficult to detect by eye but can be identified using the RR analysis feature of the Televet software. The premature complex is marked in red. The P waves are challenging to identify, but the QRS-T complex is normally shaped, suggesting that the premature complex is atrial in origin (PAC). It is followed by a compensatory (complete) pause, which theoretically is untypical for a PAC. However, such exceptions to the rule are commonly seen in practice. At lower heart rates during the recovery phase (center), the PAC (marked in red) can easily be identified and is characterized by a premature P’ wave and a normally shaped QRS-T complex. Since P waves are of variable configuration and influenced by motion artefacts, the P’ wave configuration does not obviously differ from the regular P waves. The PAC is followed by a typical non-compensatory (incomplete) pause. The third PAC seen in in this example (bottom) is characterized by a premature P’ wave, an abnormally shaped QRS-T complex indicating aberrant ventricular conduction due to the very short coupling interval, and a non-compensatory (incomplete) pause.

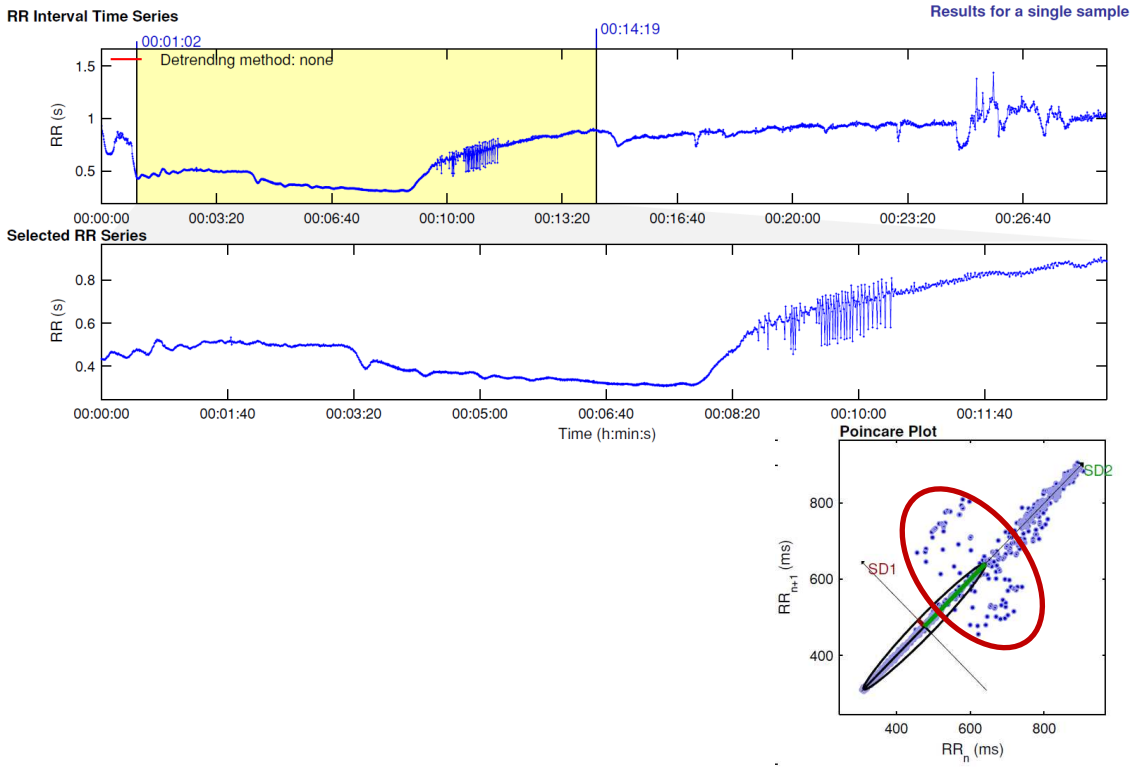


HR time series indicating a regular underlying rhythm during walk, trot and canter phases. Single sharp spikes are evident during the canter (2 solid arrows) and recovery periods (3 dashed arrows), corresponding to isolated PACs.

3.2. Cluster of PACs during recovery phase



ECG during the recovery from a high-speed treadmill exercise test. In the top segment, there are P' waves buried in the preceding T waves; they are followed by normally shaped QRS-T complexes, suggesting that the premature beats are atrial in origin (PACs). In the bottom segment, there is a P' wave with a slightly abnormal configuration, again followed by a normally shaped QRS-T complex.

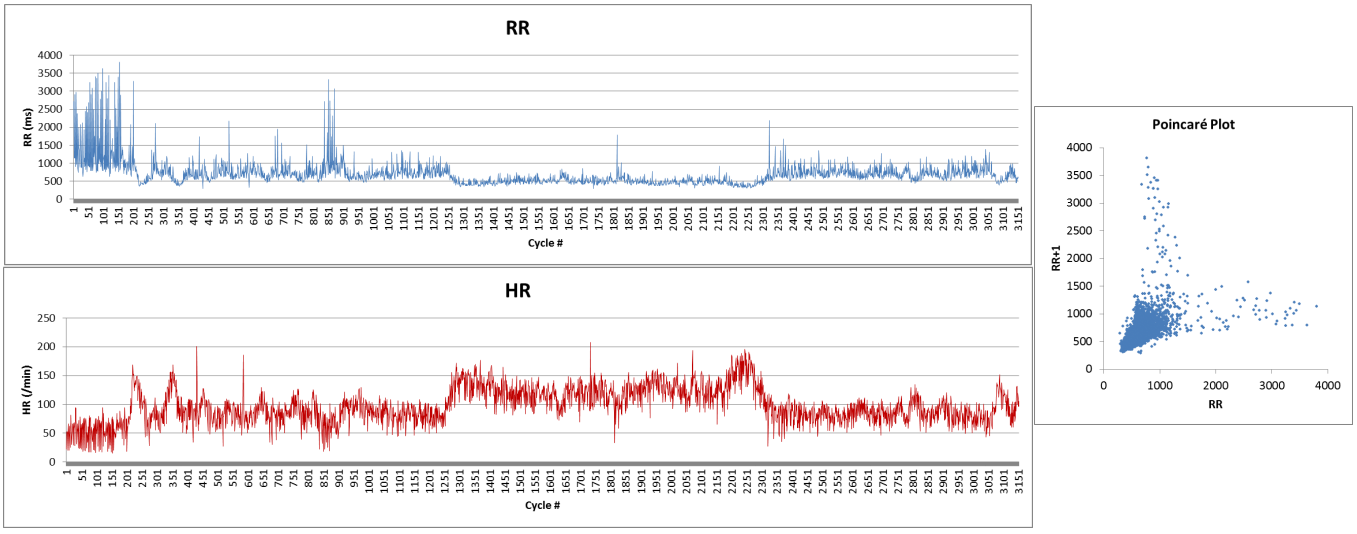


RR time series and Poincaré plot. The top RR series includes the entire recording, while the bottom segment only displays the area of interest (yellow segment), including the trot, canter and recovery phase. While the walk, trot and canter phases are characterized by very low RR variability, there is a cluster of sharp spikes during the recovery phase, corresponding to a period of 90 seconds in which at total of 25 PACs occurred. In the Poincaré plot, these beats appear at RR intervals between 600 and 800 ms and are displayed as individual datapoints remote from the diagonal line of identity along which the regular RR intervals are aligned (red circle).

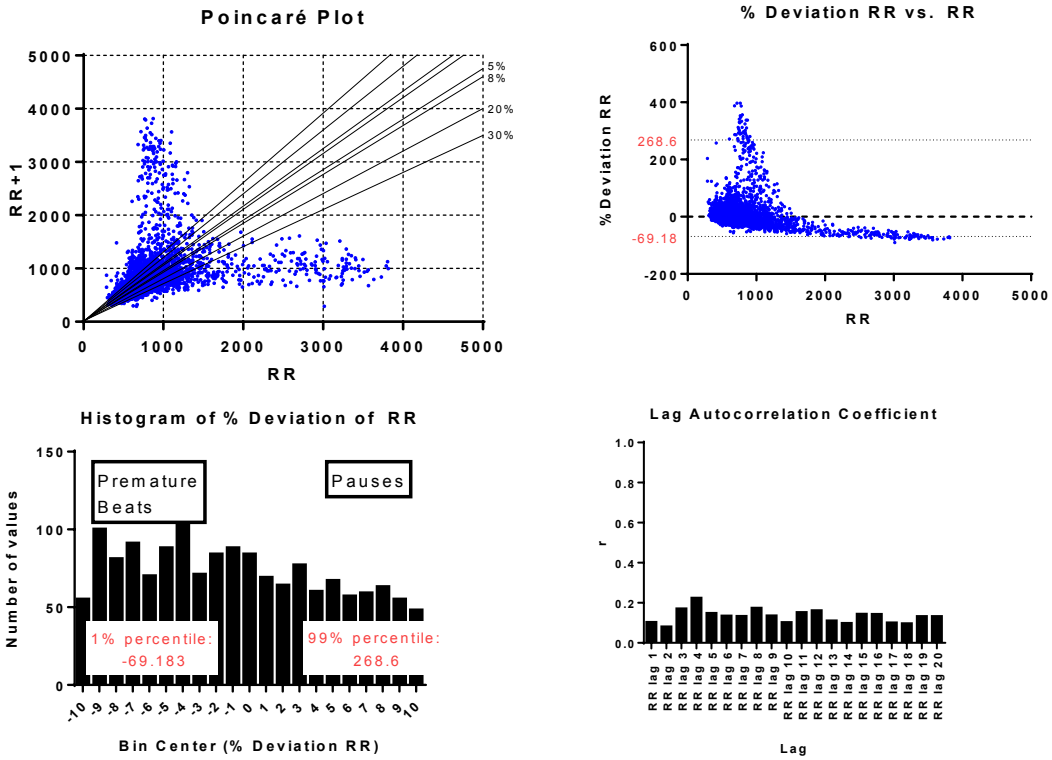
4. Atrial fibrillation

4.1. AF with normal heart rate response to exercise

ECG at rest, trot and canter. At rest, the irregularly irregular heart rhythm, the absence of a distinct P wave and the presence of smaller f (fibrillation) waves, larger F (flutter) waves and normal QRS-T complexes are indicative of atrial flutter/fibrillation. At higher heart rates, the rate becomes more regular, but close inspection of the instantaneous heart rates (corresponding to RR intervals) clearly shows that the rhythm is still chaotic. The f / F waves become less distinct at higher heart rates (due to the shorter diastolic intervals) and are obscured by motion artefacts. Occasionally, isolated F waves can mimic P waves (*). Note that depending on paper speed the Televet software does not display the black dotted lines to mark the QRS complexes; nonetheless, the tracking and the corresponding RR intervals are available and instantaneous heart rates are displayed.

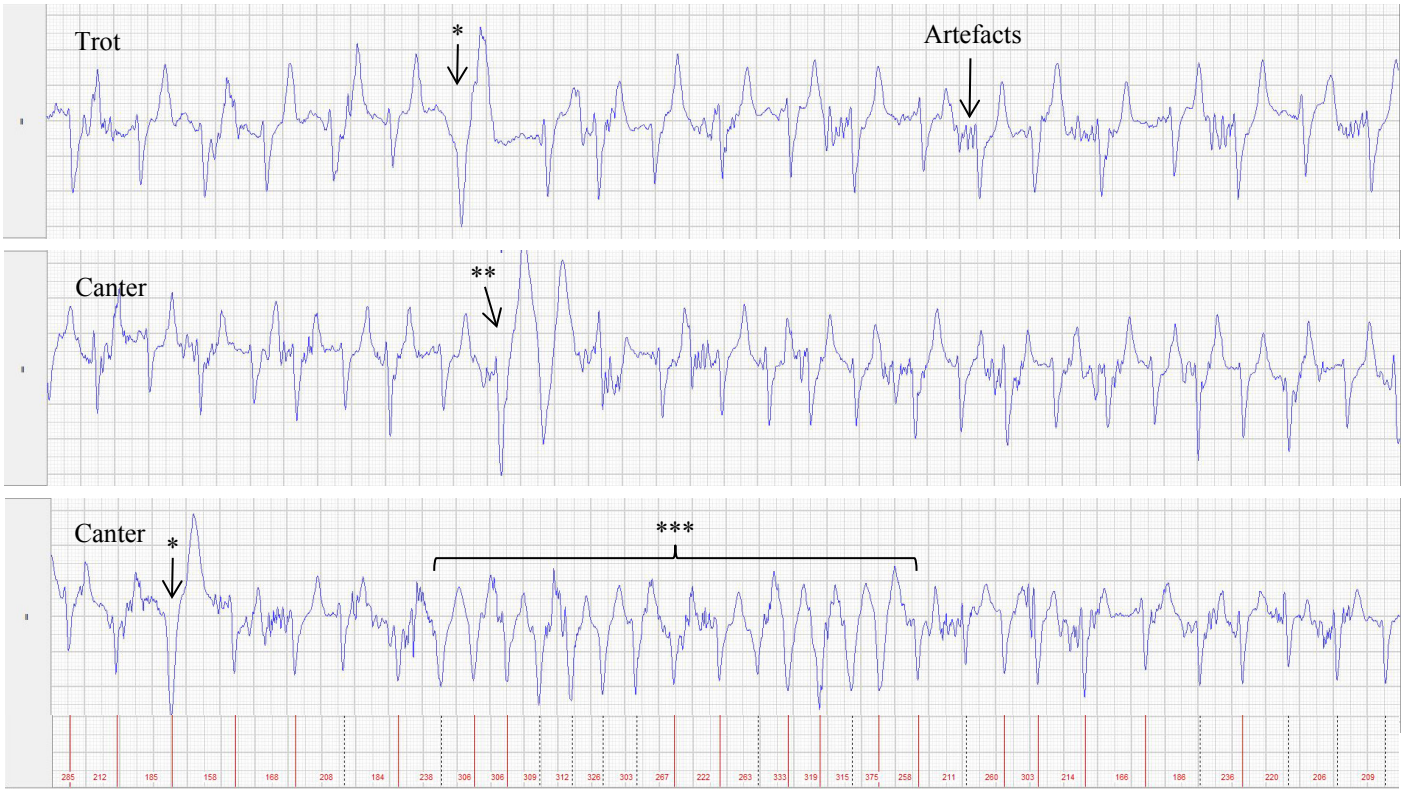


RR and HR time series and Poincaré plot. The plots indicate an irregularly irregular rhythm throughout walk, trot and canter phases. With the exception of 1 single beat, the maximum heart rate does not exceed 200 beats/min.



Detailed graphical and statistical analysis. Compared to the corresponding plots in healthy horses (see above), all plots indicate very high variability (and therefore very low correlation) between consecutive beats. The top two graphs indicate that very long cycles (i.e. 2000-3000 ms) are usually followed by short cycles (i.e. around 1000 ms), whereas shorter cycles (i.e. around 1000 ms) can be followed by short or long cycles. The histogram of the % RR deviations indicates that RR intervals vary randomly so that the % deviations are not normally distributed around 0. Similarly, the lag autocorrelations coefficients are very low over the entire range of 1-20 beats, indicating that close RR intervals do not correlate with each other.

4.2. AF with excessive heart rate response to exercise and additional ventricular ectopic beats

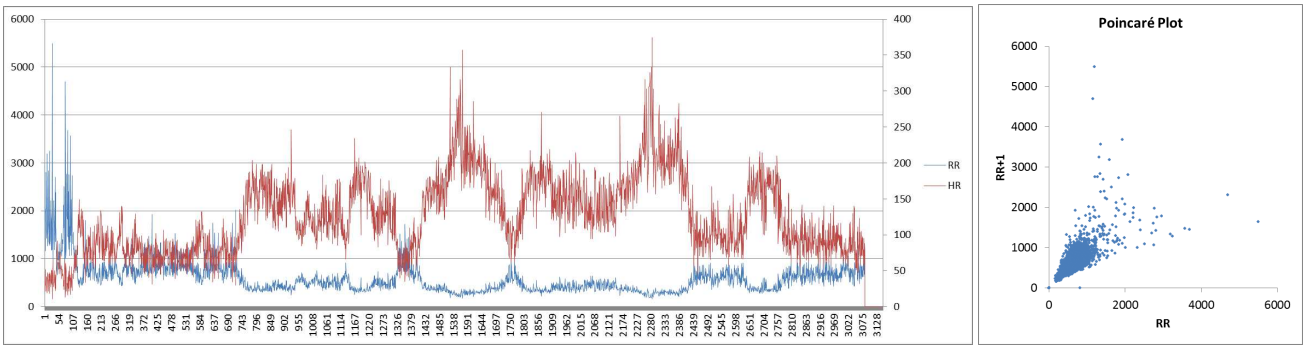


ECGs recorded during lunging exercise at a trot and canter.

Top (trot): The baseline rhythm is irregularly irregular. There is no consistent P wave. Some high-frequency motion artefacts are present. The rhythm is interrupted by a wide and bizarre complex followed by a pause, consistent with a premature ventricular complex (PVC, *).

Center (canter): A couplet of PVCs is present on top of the underlying AF (**). The coupling interval is very short, so that the QRS complex of the second PVC immediately follows the preceding T wave. If the T wave of a complex completely merges with the following premature QRS complex, this is called "R-on-T phenomenon". This is a sign of malignancy and indicates a high risk of ventricular fibrillation and sudden cardiac death.

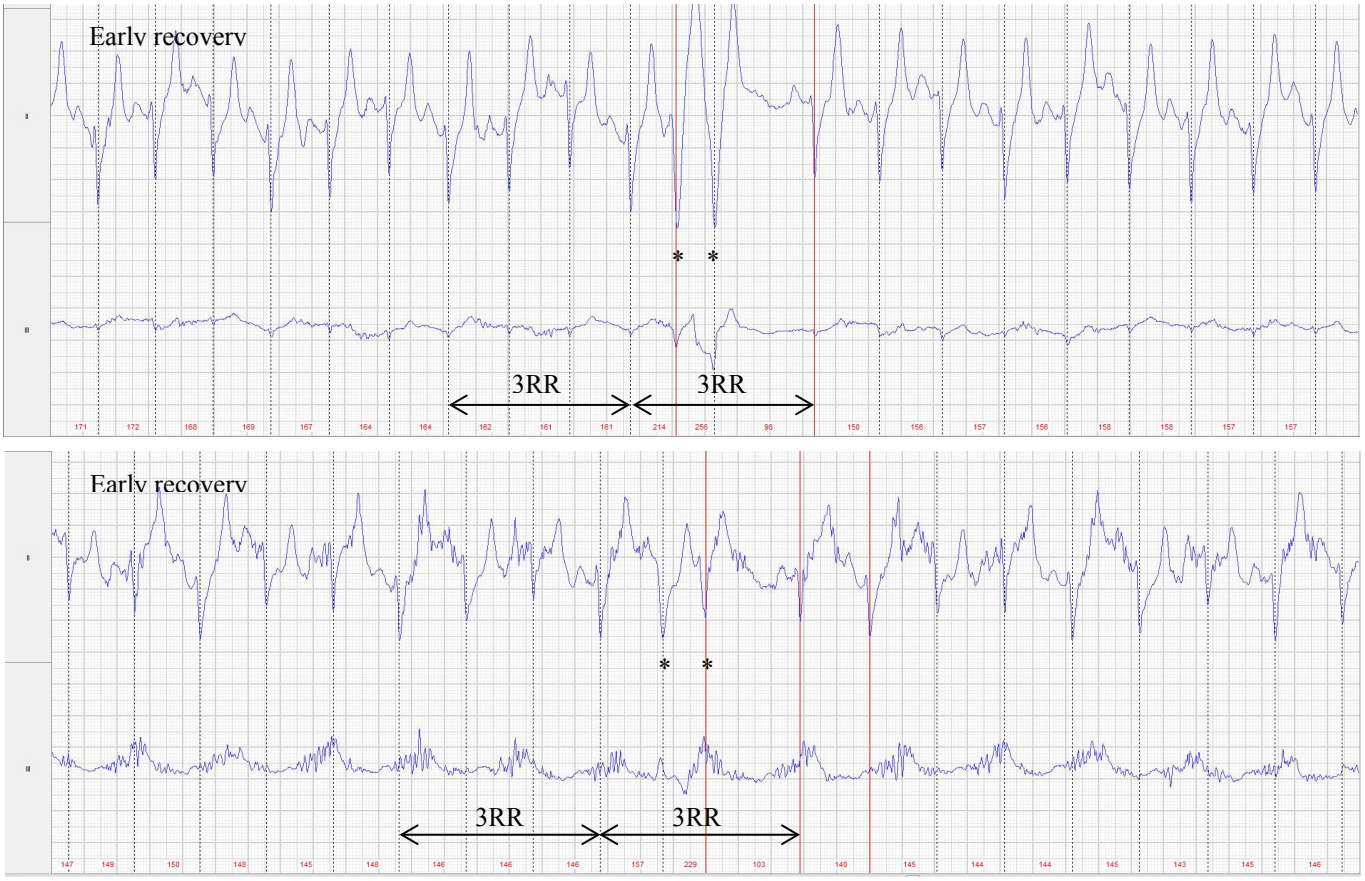
Bottom (canter): A PVC is present on top of the underlying AF (*). Subsequently, there is an episode of a very rapid rhythm well exceeding 300 beats/min and with slightly wider QRS complexes, consistent with a run of ventricular tachycardia (VT).



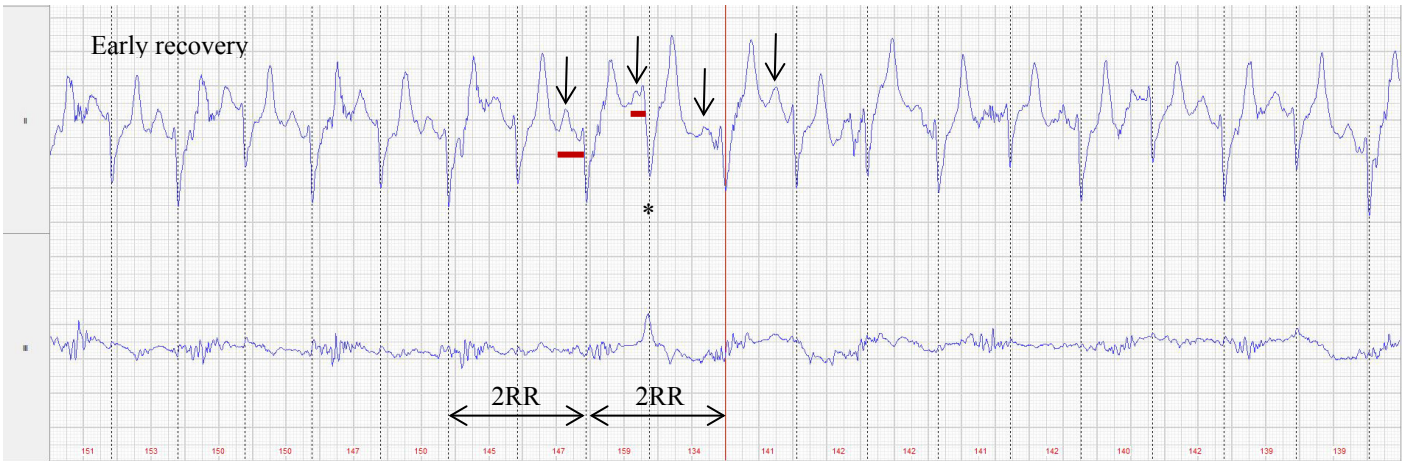
RR and HR time series and Poincaré plot. The plots indicate an irregularly irregular rhythm throughout walk, trot and canter phases. During the canter phase, the maximum heart rate exceeds 200 beats/min at two occasions with instantaneous peaks over 300 beats/min.

5. Premature ventricular complexes and ventricular tachycardia

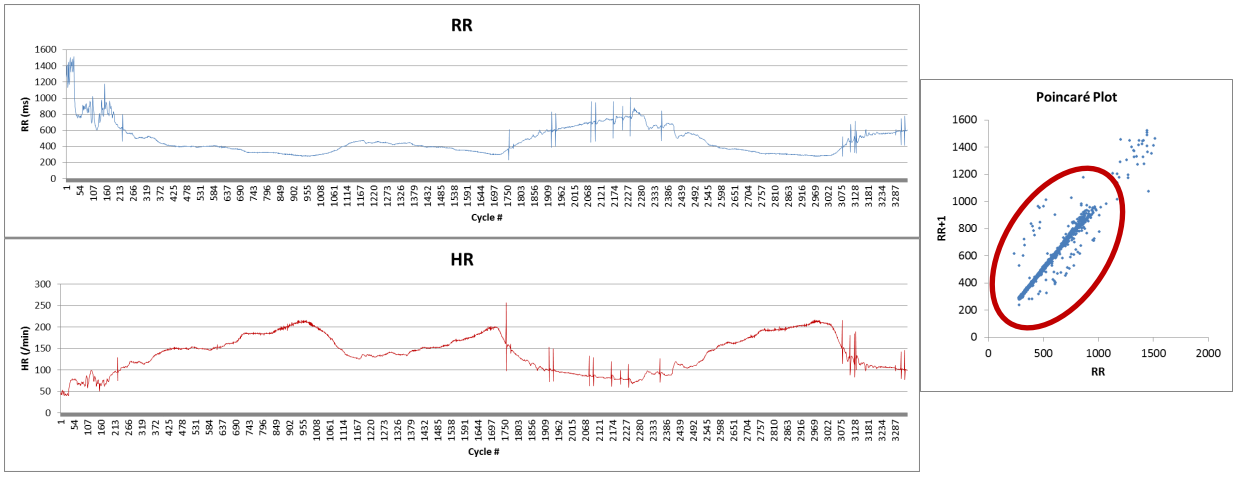
5.1. PVCs and couplets during recovery from exercise



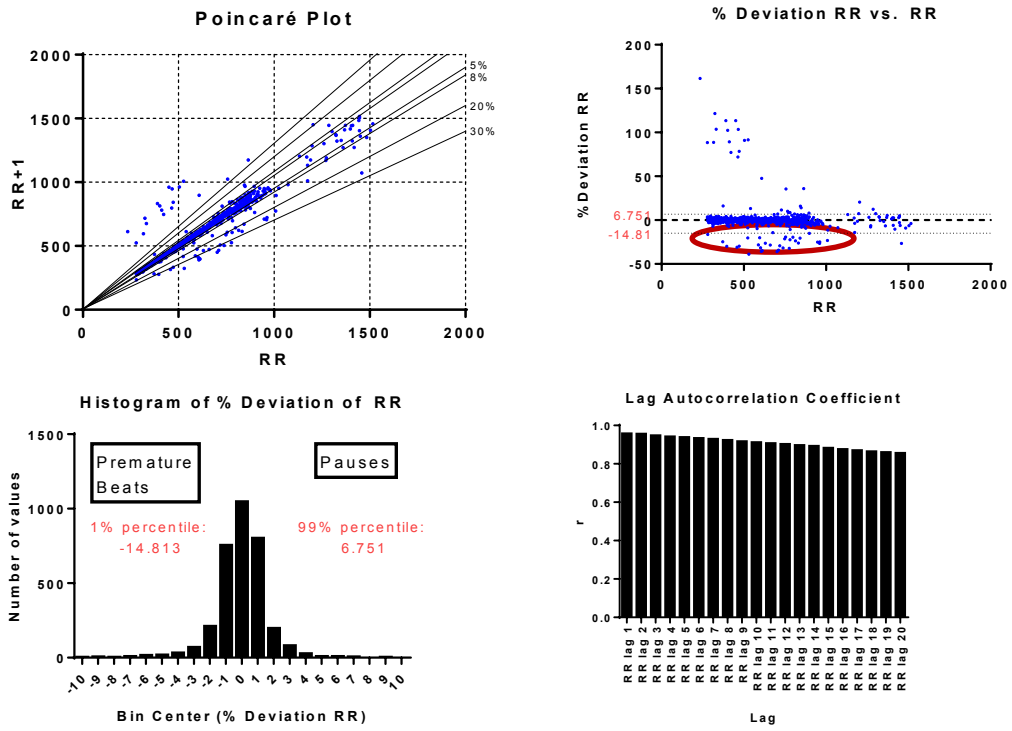
ECG recorded during the early recovery phase after a treadmill exercise test. In both segments, the regular underlying rhythm is interrupted by two consecutive PVCs (couplet; * *) followed by a compensatory (complete) pause (i.e. the underlying sinus rhythm is not interrupted and next following normal complex occurs at the expected time interval). In the top segment, the coupling interval between the two PVCs is very short, leading to a near R-on-T configuration.



In this example, the regular underlying rhythm is interrupted by a PVC (*), followed by a compensatory (complete) pause. Note that a P wave is visible immediately prior to the premature complex and that the P-P intervals are regular and not interrupted (arrows). Also note that the PQ interval of the premature beat is shorter than the regular PQ interval (red bars), suggesting that this P wave was not conducted through the AV node and is not associated with the premature beat.



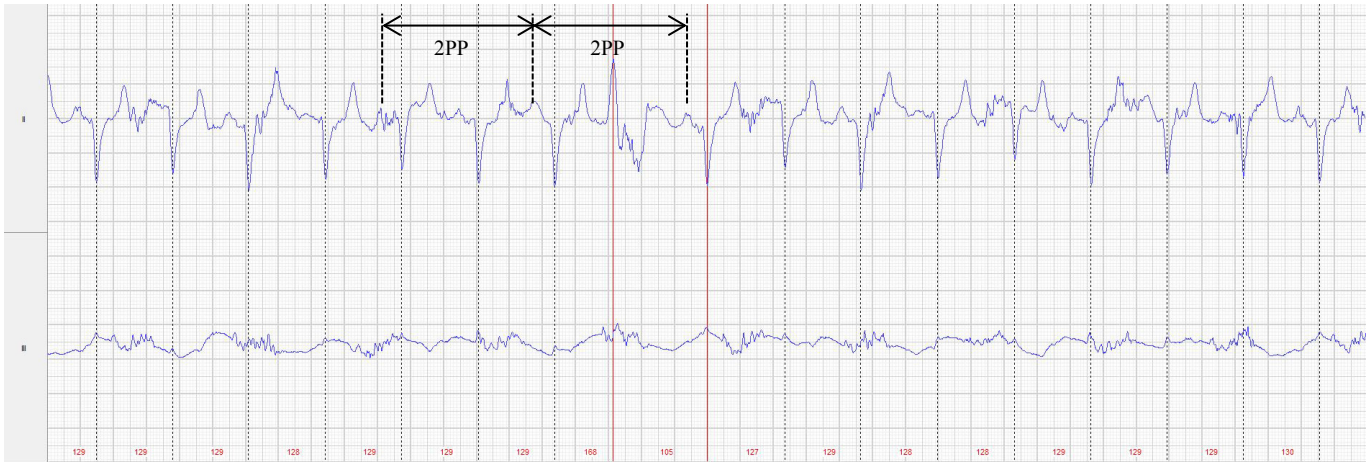
RR and HR time series indicating a regular underlying rhythm during walk, trot and canter phases. Sharp spikes are evident during the recovery periods after the warm-up and the exercise test, respectively, corresponding to the PVCs seen in the detail view. There were a total of 30 PVCs in the recovery period but none during the actual exercising periods. In the Poincaré plot, the premature beats appear at RR intervals between 400 and 800 ms and are displayed as individual datapoints remote from the diagonal line of identity along which the regular RR intervals are aligned (red circle).



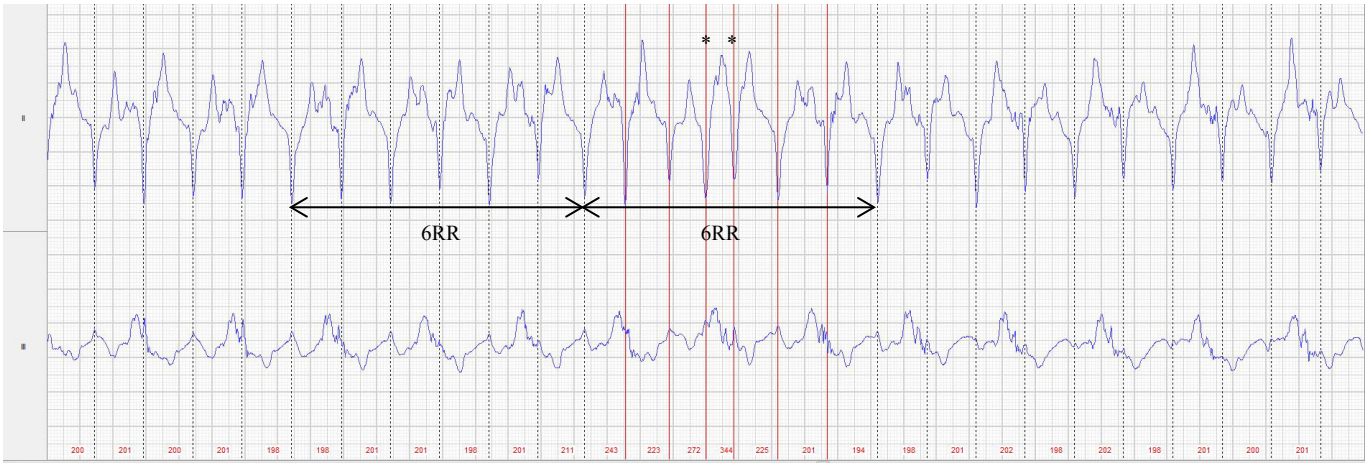
Detailed graphical and statistical analysis. Compared to the corresponding plots in healthy horses (see above), all plots indicate more variability (and therefore lower lag autocorrelation coefficients) between consecutive beats. The top two graphs indicate that premature beats primarily occur at cycle lengths between 300 and 800 ms (corresponding to heart rates between 150 and 75 beats/min), which is consistent with the findings of the detailed analyses and the findings of the RR and HR time series (see above). One 1% of RR intervals (i.e., approx. 30 beats) are > 14.81 % shorter than the preceding RR interval (supposedly the PACs). The lag autocorrelation coefficients are high (i.e. > 0.8) but decrease over the range of 1-20 beats, indicating that more distant RR intervals correlate less than more closely located intervals.

5.2. Run of VT during exercise and PVCs during recovery from exercise

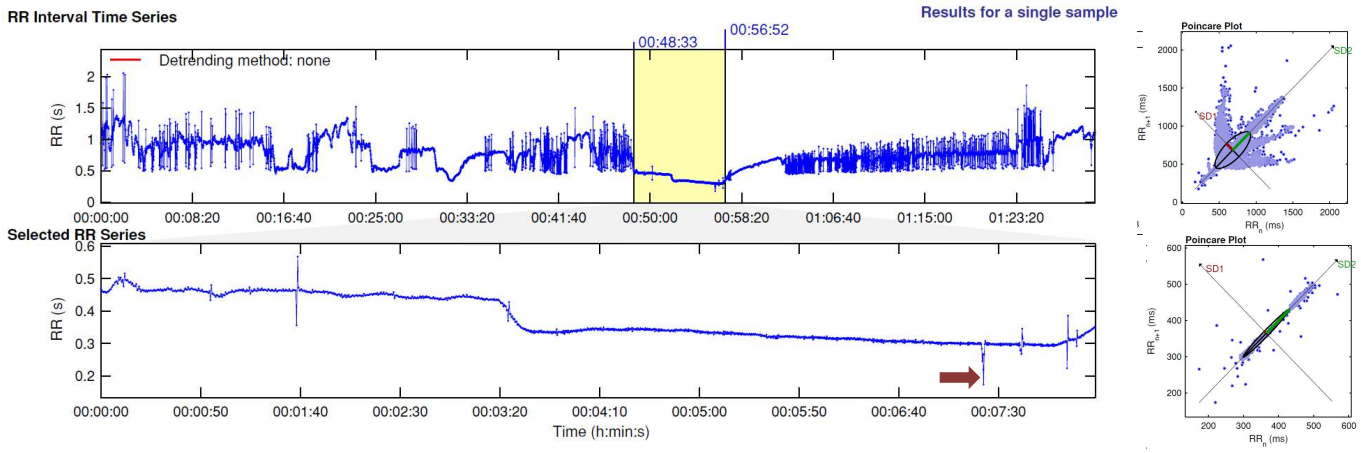
ECG recorded during the warm-up period of an exercise test at a walk. Normal sinus complexes, characterized by a normal P-QRS-T configuration, are alternating with slightly larger, premature QRS-T complexes with a different configuration (marked red by the software). There is no P wave associated with the abnormal QRS-T complexes, suggesting that they are ventricular in origin. This rhythm is termed “ventricular bigeminy”. Note that the SA nodal rhythm is not disrupted and that a regular PP interval can be traced throughout the entire ECG segment. Only every second P wave is conducted through the AV node, eliciting a normal QRS-T complex. Every other P wave is superimposed to the PVC and usually appears immediately after the premature QRS complex in the ST segment.



ECG recorded during the trotting phase of a treadmill exercise test. A PVC, characterized by a premature, abnormally shaped and abnormally oriented QRS-T complex, is interrupting the regular underlying rhythm. The PVC is followed by a compensatory (complete) pause.



ECG recorded during the final canter phase of a high-speed treadmill exercise test (at a speed of 8 m/s and an incline of 6%). The regular underlying rhythm is interrupted by a short run of ventricular tachycardia. Note that the QRS-T configuration of the premature complexes is not markedly different from the normal QRS-T complexes. However, the sudden onset and the high rate (i.e. short RR intervals) together with the fact that the underlying rhythm is not interrupted (leading to a compensatory, complete pause) suggests that this rhythm is ventricular in origin. The 3rd and 4th complex (*) occur with a very short coupling interval, suggesting a risk for R-on-T and development of ventricular fibrillation.



RR time series of the preparation and warm-up period, the exercising period, and the recovery period of a high-speed treadmill exercise test (top graph). The trotting and canter phases are selected and highlighted in yellow and displayed separately in extended view (bottom graph). This horse had a total of 1488 uniform, singular PVCs on a 24-hour Holter ECG examination. The RR time series indicates a high number of premature beats (based on the detailed analysis PVCs) during the preparation, warm-up and recovery periods, as indicated by the negative (premature beats)/positive (pauses) spikes seen on the tracing. The frequency of premature beats is markedly decreased during the trot and canter phases. However, the RR time series of the selected period (bottom graph) also indicates a few premature beats. The negative spike towards the end of the canter period (red arrow) corresponds to the run of VT identified during ECG analysis (see above). The Poincaré plot corresponding to the entire recording (top right) shows a wide-spread distribution of beats, consistent with the high number of PVCs. The Poincarè plot corresponding to the trot and canter phases (bottom right) indicates a mostly regular rhythm (with the majority of beats lined up along the diagonal line of identity), with just a few irregular beats.

REFERENCES

- Young L, van Loon G. Diseases of the heart and vessels. In: Hinchcliff KW et al. (eds.): Equine Sports Medicine and Surgery. 2nd ed. Saunders Elsevier; 2014:695-743.
- Bonagura JD, Reef VB, Schwarzwald CC: Cardiovascular Diseases. In: Reed SM et al. (eds): Equine Internal Medicine, 3rd ed. Saunders Elsevier, 2010:372-487.
- Marr CM, Bowen M (eds): Cardiology of the Horse. 2nd ed. Saunders Elsevier, 2010.
- Reef VB, Bonagura J, Buhl R, McGurrin MKJ, Schwarzwald CC, van Loon G, Young LE. Joint ACVIM/ECEIM Consensus Statement: Recommendations for Management of Equine Athletes with Cardiovascular Abnormalities. J Vet Intern Med 2014;28(3):749-761.
- Allen KJ, Young LE, Franklin SH. Evaluation of heart rate and rhythm during exercise. Equine Vet Educ 2015; doi: 10.1111/eve.12405.
- Buhl R, Meldgaard C, Barbesgaard L. Cardiac arrhythmias in clinically healthy showjumping horses. Equine Vet J 2010;42:196-201.
- Jose-Cunilleras E, Young LE, Newton JR, et al. Cardiac arrhythmias during and after treadmill exercise in poorly performing thoroughbred racehorses. Equine Vet J Suppl 2006:163-170.
- Ryan N, Marr CM, McGladdery AJ. Survey of cardiac arrhythmias during submaximal and maximal exercise in Thoroughbred racehorses. Equine Vet J 2005;37:265-268.
- Trachsel DS, Bitschnau C, Waldern N, et al. Observer agreement for detection of cardiac arrhythmias on telemetric ECG recordings obtained at rest, during and after exercise in 10 Warmblood horses. Equine Vet J 2010;42:208-215.
- Verheyen T, Decloedt A, van der Vekens N, et al. Ventricular response during lungeing exercise in horses with lone atrial fibrillation. Equine Vet J 2013;45:309-314.